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Conservation of binding site specificity of three yeast DNA binding proteins

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Sequence specific binding of protein extracts from 13 different yeast species to three oligonucleotide probes and two points mutants derived from *Saccharomyces cerevisiae* DNA binding proteins were tested using mobility shift assays. The probes were high affinity binding sites for GRF1/RAP1/ABF1 and CP1/CPF1. Most yeasts in the genus *Saccharomyces* showed specific binding to all three probes and also displayed similar sequence requirements when challenged by molar excesses of mutant probes. The affinities for the probes varied amongst the other yeasts tested, but in general, CPF1 binding activity was the most widespread, while the other two were more limited.

Transcription factor; Protein–DNA interaction; Mobility shift assay

1. INTRODUCTION

During the last few years, several abundant sequence specific DNA binding proteins have been identified in *Saccharomyces cerevisiae* [1]. These proteins fulfill various regulatory and/or structural functions and are currently the subjects of intense investigation. Although exact structure–function relationships have been established for some of the proteins, others need further analysis. One approach to address this problem could be to investigate the occurence of selected sequence specific binding proteins in extracts from a variety of different yeasts, using as probes binding site sequences derived from *S. cerevisiae*. We have decided to explore this route and the yeast species were chosen to represent a range of relatedness to *S. cerevisiae* in order to also gain insight into evolution of DNA binding proteins.

We chose three *S. cerevisiae* DNA binding proteins namely GRF1/RAP1 [2,3], ABF1 [2] and CP1/CPF1 [4,5] on the basis of their abundance and well-characterised sequence requirements. For each protein we used as probe a double-stranded oligonucleotide containing a high affinity binding site (wild-type), while for the first two proteins we also used point mutants which severaly affect their binding by the corresponding *S. cerevisiae* proteins. By including molar excesses of unlabeled wild-type or mutant oligonucleotides in mobility shift assays, we hoped to gain information regarding both the presence and specifity of corresponding DNA binding proteins in the various yeasts.

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2. EXPERIMENTAL

2.1. *Yeasts*

The yeast strains used as sourse of protein extracts are listed in Table I and are maintained in the culture collection of this department.

2.2. *Oligonucleotides*

The oligonucleotides were a kind gift from A.R. Buchman, Pennsylvania State University, USA [6,7].

2.3. *Preparation of extracts and mobility-shift assays.*

The yeasts were grown with shaking at 30°C in 100 ml YPD broth to mid-logarithmic phase. Lysis was achieved by vortexing with glass beads and preparation of S100 extracts were done as described [8]. Oligonucleotide probes were labeled by filling in the 5' overhanging ends with Klenow polymerase and [^{32}P]dATP. Fifty μg of protein extract from each yeast was used per assay, done essentially as in Buchman et al. [2]. Assays contained 10^4 cpm (0.05–0.1 ng) of labeled probe, 1 μg of herring sperm DNA as non-specific competitor and a 50-fold molar excess of unlabeled oligonucleotide when required. The gels (4% polyacrylamide) were dried after electrophoresis and exposed to X-ray film (Cronex 4). Band intensities were quantified by scanning the films with a Hoefer GS300 densitometer and accompanying software. The numbers in Table II were calculated as follows: $(s/n) \times 100$, where s is the fraction of radioactivity in the complexed band in the presence of unlabeled oligonucleotide competitor and n is the same fraction in the absence of oligonucleotide competitor.

3. RESULTS AND DISCUSSION

Most yeasts in the genus *Saccharomyces* (fig.1, lanes 1–5) showed binding to all three targets, albeit with different affinities. In all three cases, *S. dairensis*, *S. unisporus* and *S. servazzii* showed similar shifts in mobility which indicate proteins of similar size, while *S. kluyveri* displayed a smaller shift in mobility. Competition with unlabelled wild type oligonucleotides (Table II) indicated that the binding of *S. kluyveri* to the RAP1 binding site (ENO1) and *S. dairensis* to the CPF1 target (GAL2) were non-specific. Based on partial rDNA se-

Table I

Yeast species and abbreviations

Species	Abbreviation	Strain
Saccharomyces cerevisiae	*S. cerevisiae*	*CSIR Y2*
Saccharomyces kluyveri	*S. kluyveri*	*CBS 3082T*
Saccharomyces dairensis	*S. dairensis*	*CBS 421T*
Saccharomyces unisporus	*S. unisporus*	*CBS 398T*
Saccharomyces servazzii	*S. servazzii*	*CBS 4311T*
Kluyveromyces marxianus	*K. marxianus*	*CBS 1556*
Debaryomyces hansenii	*D. hansenii*	*CSIR Y510*
Debaryomyces polymorphus	*D. polymorphus*	*CSIR Y587*
Debaryomyces melissophilus	*D. melissophilus*	*CSIR Y903*
Pichia stipitis	*P. stipitis*	*CBS 5773T*
Candida utilis	*C. utilis*	*ATCC 9256*
Schizosaccharomyces pombe	*Schiz. pombe*	*CBS 356T*
Galactomyces geotrichum	*G. geotrichum*	*CBS 772.71*
Schwanniomyces occidentalis	*Schw. occidentalis*	*CSIR Y993*

quences, Kurtzman and Robnett [9] constructed a phylogenetic tree of *Saccharomyces*, *Debaryomyces*, *Schwanniomyces* and *Schizosaccharomyces*. On the *Saccharomyces* branch of this tree, *S. servazzii* and *S. unisporus* clustered close together, with *S. dairensis* somewhat further away, while *S. kluyveri* stood away from the rest of the genus. The same tendency was observed using chromosome separations [10] and total DNA hybridization within the genus [11].

As juged from the binding and competition results, *K. marxianus* is the closest relative to *Saccharomyces*, followed by *D. hansenii* (Fig. 1. and Table II, lanes 6,7). In a recent study, Dorsman et al. [12] reported the existence of proteins in *K. lactis* that recognised targets for GF1 and GF2 (which have subsequently been proved to be identical to ABF1 and CPF 1, respectively [13]). *K. lactis* and *K. marxianus* are so closely related that they have been synonymised in the past, although they have been separated again [14].

All three proteins analyzed in this study are multifunctional factors involved in activation/repression of transcription, regulation of replication as well as having structural roles. Our results indicate that the binding sites for RAP1 and ABF1 are less well conserved than that of CPF1, since virtually all yeasts tested contain

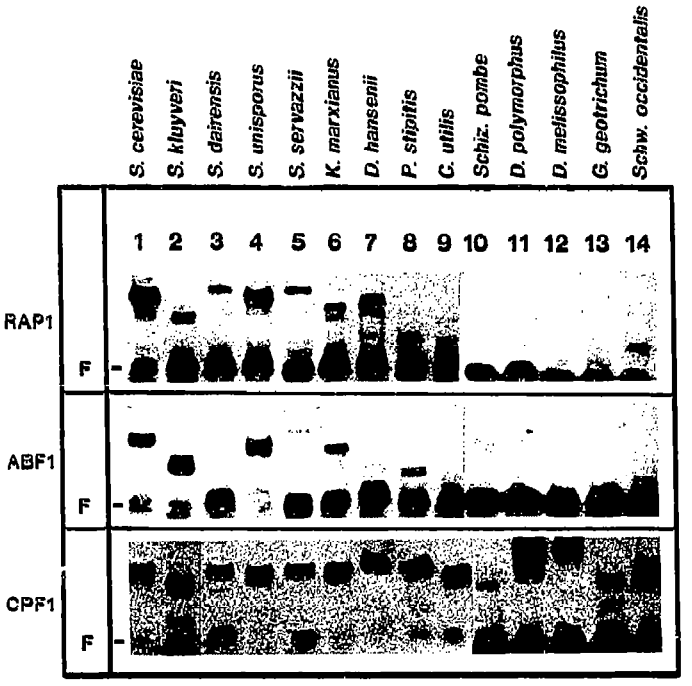

Fig. 1. Mobility-shift assays of yeast crude extracts. Three different probes were used in the horizontal panels: ENO1 was used for detection of RAP1, SPT2 for ABF1 and GAL2 for CPF1. F denotes the position of the free probes.

activities binding to the GAL2 oligonucleotide. Bram and Kornberg [15] found an activity in human cells that recognised the consensus CPF1 binding site at the *S. cerevisiae* CDE1 element, which forms part of the centromere. It is therefore all the more surprising that *S. kluyveri* and *Schiz. pombe* showed very little binding (Fig. 1) to the CPF1 binding site, and that *S. dairensis* bound the target non-specifically.

Very little is known about the nuclear organization of yeasts other than *S. cerevisiae*, an exception being *Schiz. pombe* [16,17]. The *Schiz. pombe* centromere is much larger and more complex than that of *S. cerevisiae*, and its binding site for structural and regulatory proteins may thus also be different. It is significant that the sequence preferences as judged by com-

Table II

Competitive binding of yeast protein extracts to oligonucleotides

	Yeast strain*													
Probe	1	2	3	4	5	6	7	8	9	10	11	12	13	14
ENO1	44	100	37	23	55	31	32	NB	NB	NB	NB	NB	NB	70
ENO1mtA	100	100	100	100	96	100	100	NB	NB	NB	NB	NB	NB	100
SPT2	20	66	NB	28	31	33	NB	87	NB	NB	NB	NB	NB	NB
SPT2mtA	99	98	NB	100	86	100	NB	95	NB	NB	NB	NB	NB	NB
GAL2	44	46	100	36	22	38	77	86	54	88	53	55	72	72

NB = no binding.

* The strain numbers are the same as in Fig. 1.

petition with mutant oligonucleotides (Table II), are generally closely related to that found in *S. cerevisiae*. The results obtained in this study may lead to the identification and cloning if genes coding for DNA binding proteins in a variety of yeasts, which in turn could shed light on the mechanisms involved in the evolution of these proteins and their cognate binding sites.

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