Isolation of CENIX and CENXII from _Saccharomyces cerevisiae_

Kristina Wustinger and Walter Spevak*
Ernst Boehringer Institut für Arzneimittelforschung, Dr. Boehringerstrasse 5-11, A-1121 Vienna, Austria

Received May 26, 1993; Accepted June 9, 1993

The disruption of the MSI1 gene (1) in wild type strain DBY747 leads to instability of 2µ derived episomal plasmids. After growing for 24 hours under nonselective conditions the wild type strain has a plasmid retention rate of about 70%, whereas the msil mutant strain shows a retention rate of less than 5% (2). The mutant strain was transformed with a 2µ based gene library (3) and eight high copy suppressors of the plasmid loss phenotype were isolated.

The plasmid inserts were sequenced and used to search the GenEMBL sequence database. Six of these sequences are identical to previously isolated centromeric sequences. No sequences similar to two suppressors, pMSS3 and PMSS17, were found in the database. Nevertheless, these suppressors also contain the consensus elements which are characteristic of centromeres, as shown in Figure 1 (4). To date 13 of the 16 yeast centromeres have already been isolated and analysed. The centromeres of chromosome VIII, IX and XII have not yet been described. A chromosome blot analysis indicates that we have isolated the centromeric regions derived from chromosome IX and XII. The CDEI motif is well conserved in both sequences, followed by the AT rich stretch of the CDEII element. The consensus requirements for the CDEIII elements are also fulfilled. Interestingly in the core region of CDEIII, a T at position 11 is replaced by an A in CENXII. The sequence for CENIX exhibits two changes in comparison to the consensus. First, T at position 3 is replaced by a G, and three As at positions 23 –25 are replaced by three Ts in the CDEIII element. An _in vivo_ assay for centromeric function was performed. The DNA regions shown in Figure 1 were isolated by PCR and cloned into the YRP plasmid pKE5 (5). The isolated CENIX and CENXII sequences confer the same stability to the pKE5 plasmid as does CENVI, and hence clearly have centromeric function.

ACKNOWLEDGEMENT

We thank Dr J.H. Hegemann for providing the plasmid pKE5.

REFERENCES


Figure 1. Sequences of pMSS3 and pMSS17 that encode the CENIX and CENXII, respectively. The CDEI, CDEII and CDEIII elements are indicated. # consensus sequence, as published (4). R, purine.

* To whom correspondence should be addressed