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Autoregulation of expression of the yeast Dbp2p 'DEAD-box' protein is mediated by sequences in the conserved *DBP2* intron

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for its length, 1001 nucleotides, which is twice that of any other known intron in *S.cerevisiae* (Rymond and Rosbash, 1992). These unusual features suggest that the

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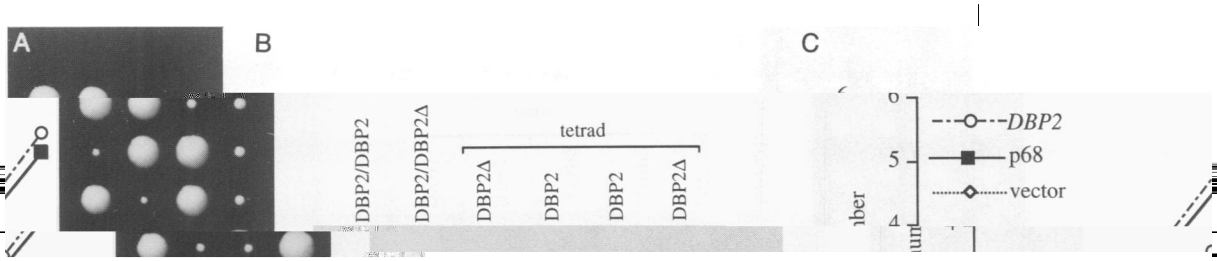
participate in regulation of *DBP2* expression, or both. The

intron does not contain conserved sequences or long open



growth at 35°C for 4 days. (**B**) Western blot probed with MaD1 (a, parental diploid; b, heterozygous diploid; c–f, haploid progeny of a heterozygous

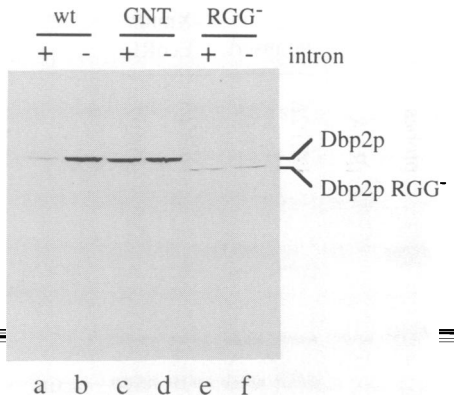


Fig. 4. Western blot probed with KT3 antibody to detect exogenous Dbp2p in a wild-type strain containing plasmids expressing *DBP2* intron. Lanes a and b: wild-type *DBP2*; lanes c and d: GNT mutant *DBP2*; lanes e and f: RGG⁻ mutant *DBP2*. RGG⁻ Dbp2p runs faster than wild-type and GNT point-mutant protein because it lacks 49

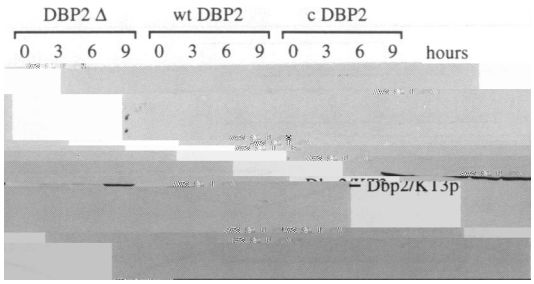
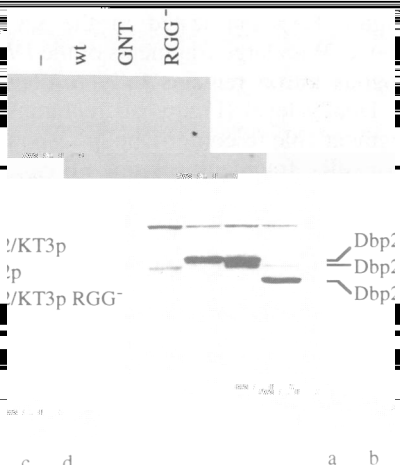


Fig. 6. Western blot probed with KT3 showing that endogenous Dbp2p can suppress exogenous *DBP2* expression. KT3-tagged wild-type Dbp2p was expressed from the *GAL1* promoter in strains with the null *(DBP2Δ)*, wild-type *(DBP2)* or intronless allele *(cDBP2)* at the chromosomal *DBP2* locus (yIB12/1, 12/2 and 37, respectively). The plasmid (pIG85) copy of *DBP2* contains the *DBP2* intron. Exogenous *DBP2* production was induced by growing cells in galactose for the



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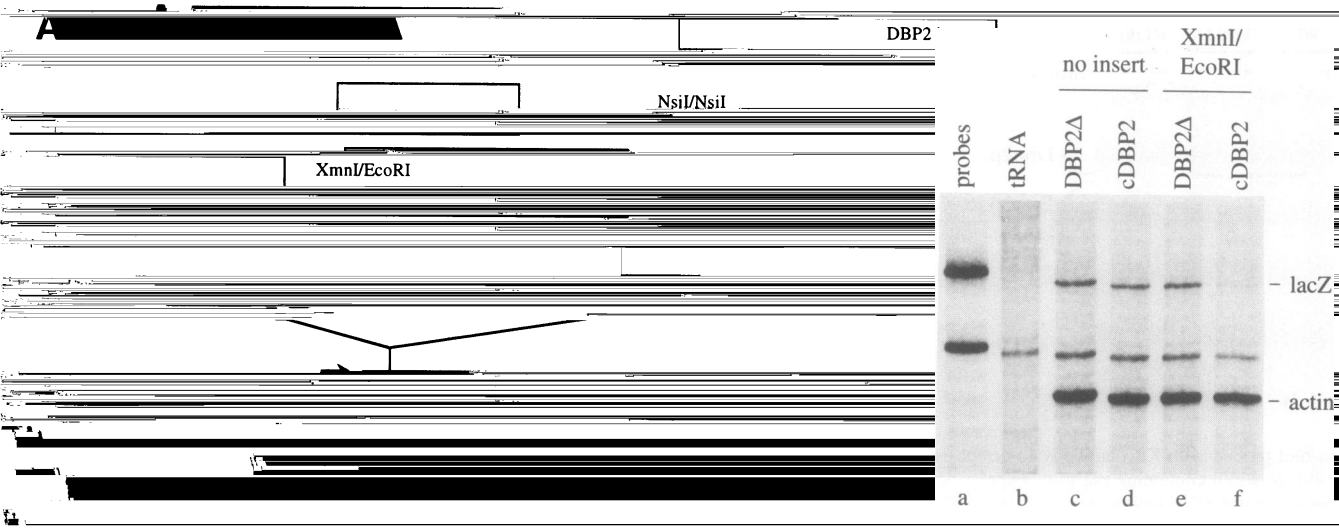

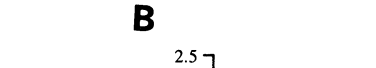

Fig. 9. Dbp2p feedback decreases heterologous reporter expression at

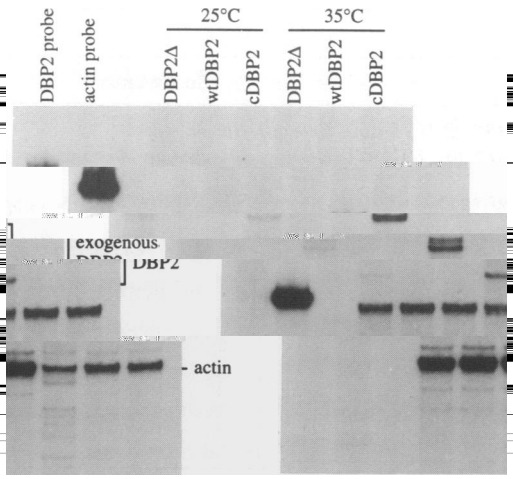

Fig. 10. Dbp2p feedback does not lead to accumulation of *DBP2*

processing event (Fournier and Maxwell, 1993; Sollner-Webb, 1993).

The most economical model predicts that autoregulation of *DBP2* expression exploits one of the general functions of the *DBP2* gene. This is difficult to test until the normal function of the *DBP2* gene is known. Northern blotting of the *DBP2* null strain suggests that there is a defect in splicing of the *RP28* intron, although several other introns are spliced normally (unpublished data). Since pre-mRNA secondary structure can strongly influence the efficiency with which splicing signals are used (Eperon *et al.*, 1988; Chebli *et al.*, 1989; Deshler and Rossi, 1991; Goguel *et al.*, 1993), loss of an RNA helicase could readily produce a rather selective splicing defect, and autoregulation of production of such a helicase would amount to a simple

The mechanism of *DBP2* autoregulation is not clear from our data. Transcriptional repression or pausing is

Table I. Yeast strains used in the present studies

Strain	Genotype	Source/cross
ASZ3	**a**/α *ade2-/ade2-1 ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 his3-11,15/his3-11,15 can1-100/can1-100*	P.Linder
vIB12	**a**/α *ade2-1/ade2-1 ura3-1/ura3-1 leu2-3.112/leu2-3.112 trp1-1/trp1-1 his3-11.15/his3-11.15 can1-100/ can1-100 DBP2/dbp2Δ1::URA3*	ASZ3 + pIB3
vIB15	**a**/α *ade2-1/ade2-1 ura3-1/ura3-1 leu2-3.112/leu2-3.112 trp1-1/trp1-1 his3-11.15/his3-11.15 can1-100/ can1-100 DBP2/cDBP2*	ASZ3 ± pIB8
vIB36	**a**/α *ade2-1/ade2-1 ura3-1/ura3-1 leu2-3.112/leu2-3.112 trp1-1/trp1- his3-11.15/his3-11.15 can1-100/ can1-100 cDBP[redacted]2/cDBP2*	vIB15[a]
vIB12/2	α *ade2-1 ura3-1 leu2-3.112 trp1-1 his3-11.15 can1-100 DBP2*	vIB12
Cy105	**a** *ade2 ura3-52 trp1-289 leu2-3.112 gre4 prp9-1*	P.Legrain

casamino acids for trp and ura selection; SC minus relevant amino acids for selection of other markers (Guthrie and Fink, 1991). For induction of the *GAL1* promoter, cells were transferred directly from 2% glucose to 2% galactose-containing media. Two-step gene replacement was done by selecting on ura$^-$ medium for integration of pIB8 linearized at codon 496 (the intron splits codon 425) followed by counterselection on medium containing 5-fluoro-orotic acid. Construction of the *cDBP2* and *DBP2*Δ strains was verified by Southern blotting.

Northern blotting and RNase protection

The Northern blots were probed with fragments corresponding to the last 150 bp of the *DBP2* open reading frame (Figure 3A) and the last 0.9 kb of the actin open reading frame (Figure 3B). *DBP2* RNA expression was quantitated by phosphorimager and normalized to actin.

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