$\Sigma(p_iX_i)]^2\}$, where p_i and X_i are the frequency and the mean, respectively, of chromosome lines homozygous for class i of a polymorphic site, and $J_i = np_i$, then $\Sigma\{p_i[X_i - \Sigma(p_iX_i)]^2\} = [1 - \Sigma(pi)^2]\,[\sigma_c^2 + MS_{L(M)}/(NJ)]$. To account for the variance ($MS_{L(M)}$) due to the chromosome line effect and sampling error, $\Sigma\{p_i[X_i - \Sigma(p_iX_i)]^2\} = [1 - \Sigma(p_i)^2]\,\sigma_c^2$. Assuming strictly additive allelic effects, the additive genetic variance associated with a polymorphic site of r classes (that is, r alleles at one locus) is $\sigma_A^2 = 0.5\,\Sigma\{p_i[X_i - \Sigma(p_iX_i)^2\}$ [O. Kempthorne, *Introduction to Genetic Statistics* (Wiley, New York, 1957), pp. 318–324]. With the above relation, we can show $\sigma_A^2 = 0.5\,[1-\Sigma(p_i)^2]\,\sigma_c^2$, where σ_c^2 was estimated on the basis of the model described in (*13*). The same procedure was used to estimate the additive genetic variance associated with haplotypes and the sex*site interaction effect of a polymorphic site.

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17. At *ASC*, large insertions (>0.5 kb) as a class were associated with quantitative variation in bristle number (actually, an average reduction in bristle number), but each insertion was individually rare. The class of large insertions in the *sca* region was not significantly associated with bristle number variation among the second chromosome lines. The contrast in the nature of the molecular variants associated with phenotypic variation in these two regions may reflect an inherent difference in the loci or may be a consequence of the reduction in the amount of DNA sequence polymorphism (not insertional polymorphism) found in regions of low cross-over frequency per physical length [M. Augadé *et al.*, *Genetics* **122**, 607 (1989)].
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23. With complete dominance, the genetic variance associated with a diallelic polymorphic site is given by $\sigma_G^2 = 2q(1 + q)\sigma_A^2$, where q is the gene frequency of the recessive allele and σ_A^2 is the additive genetic variance (*2*). For a given gene frequency and effect at a diallelic locus, the minimal variance occurs when the recessive allele has the lower frequency, and the maximal variance occurs when the recessive allele has the higher frequency. For example, estimates of genetic variance in abdominal bristle number attributable to *Eco* RI(6.5) polymorphism with complete dominance range from 0.038 to 0.35 (compared to 0.112, assuming strict additivity). For the range of gene frequencies and effects observed, the estimated variance, assuming additivity, could be over- or underestimated by a factor of 3.
24. The correlation between the effect (a) attributable to a polymorphic site and the effect α due to the actual molecular variant with which the site is associated is given by $\alpha = \{[p(1-p)]/D\}a$, where p is the frequency of the polymorphic site and D is the linkage disequilibrium between the molecular variant directly causing the bristle number effect and the polymorphic site associated with it. Because $D \leqq p(1-p)$, then $\alpha \geqq a$; that is, the estimated effect attributable to a polymorphic site is an underestimate of the effect of the actual variant with which the site is associated.
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RNA14 and RNA15 Proteins as Components of a Yeast Pre-mRNA 3′-End Processing Factor

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Most eukaryotic pre–messenger RNAs are processed at their 3′ ends by endonucleolytic cleavage and polyadenylation. In yeast, this processing requires polyadenylate [poly(A)] polymerase (PAP) and other proteins that have not yet been characterized. Here, mutations in the *PAP1* gene were shown to be synergistically lethal with previously identified mutations in the *RNA14* and *RNA15* genes, which suggests that their encoded proteins participate in 3′-end processing. Indeed, extracts from *rna14* and *rna15* mutants were shown to be deficient in both steps of processing. Biochemical complementation experiments and reconstitution of both activities with partially purified cleavage factor I (CF I) validated the genetic prediction.

The pre-mRNAs of the yeast *Saccharomyces cerevisiae* are processed at their 3′ ends to produce transcripts ending with a poly(A) tail of about 70 adenosine residues [reviewed in (*1*)]. Yeast RNA 3′-end formation has been reconstituted in vitro (*2*) and shown to be similar to that in mammals (*3*). It consists of two reactions: endonucleolytic cleavage of the pre-mRNA and subsequent polyadenylation of the upstream fragment. Correct cleavage of the precursor occurs in vitro by combination of two partially purified factors, CF I and CF II, whereas polyadenylation of the upstream fragment requires CF I, polyadenylation factor I (PF I), and PAP itself (*4*). Thus, CF I is required for both steps of processing. Thus far, only the gene coding for PAP has been identified and shown to be essential for cell viability (*2*, *5*).

A selective screen for *S. cerevisiae* mutants that are sensitive to cordycepin (3′-deoxyadenosine) at 22°C and are temperature-sensitive at 37°C has revealed two genetically independent mutants called *rna14* and *rna15* (*6*). In these mutants, the steady-state concentration of the polyadenylated mRNAs was shown to rapidly decrease at the nonpermissive temperature (37°C), with a shortening of the poly(A) tails. This was also observed with a well-known RNA polymerase II mutant, *rpb1-1*. Because transcriptional activity was not substantially altered in *rna14* and *rna15*, these phenotypes have been interpreted as an impairment of mRNA stability (*6*). However, a defect in the maturation of pre-mRNA 3′ ends could also explain the mutant phenotypes. The latter explanation would mean that no new mature mRNA is produced, which is consistent with the phenotypes observed. Genetic and biochemical evidence is provided in this report that strongly supports the involvement of RNA14 and RNA15 proteins in 3′-end processing of pre-mRNAs; more precisely, as components of CF I.

If *RNA14*- or *RNA15*-encoded proteins are involved in the maturation of pre-mRNA 3′ ends, they may physically or functionally interact with PAP. This interaction can be assayed genetically through the analysis of double mutants of their genes: The combination of these mutations may show a synergistic interaction and lead to cell death under any conditions of growth (*7*). We thus introduced *rna14-1* or *rna15-1* mutations into a strain carrying a chromosomal disruption of the *PAP1* gene, rescued by the p*PAP1* plasmid, which contains the wild-type *PAP1* gene and the *URA3* marker. These strains (*8*) were then transformed with an *ADE2*-marked plasmid carrying the temperature-sensitive allele *pap1-5* (p*Apap1-5*) (*9*). Because of the presence of the *rna14-1* or *rna15-1* alleles, these strains are strictly temperature-sensitive but can grow at the permissive temperature (24°C) on a selective medium that allows the maintenance of the plasmids (Fig. 1A, sectors 1 and 6). However, the strains were inviable once the p*PAP1* plasmid was lost on selective medium containing 5-fluoroorotic acid (5-FOA) (*10*), even at the low temperature (Fig. 1B, sectors 1 and 6). Because on this medium the essential functions of *RNA14*, *RNA15*, and *PAP1* genes were only provided by their respective mutant alleles, we concluded that the observed lethality was due to the enhancement of the conditional defect that each of the mutants carries on its own. This conclusion was strengthened by the fact that the synergistic lethality was reversed when we introduced the plasmid-borne *RNA14* wild-type gene into the *rna14-1 pap1-5* mutant context but not when we introduced the *RNA15* gene (Fig. 1B, compare sectors 2 and 3, respectively). For the same reasons, an *RNA15*-containing plasmid allowed the *rna15-1 pap1-5* mutant to grow on 5-FOA medium, whereas transformation with the *RNA14* gene did not (Fig. 1B,

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sectors 4 and 5, respectively).

One possible explanation for the synergistic lethality of the double mutants is that the RNA14 and RNA15 proteins participate with PAP in 3′-end processing of mRNAs. To assay this possibility in vitro, we made extracts from strains harboring either the *rna14* or the *rna15* mutation (*11*). We first analyzed the cleavage reaction in conditions that allowed cleavage of the precursor only (*12*). Under these conditions, the *CYC1* precursor (Fig. 2, lane 1) was accurately cleaved in vitro in the wild-type extract, as previously shown (*2*), generating the upstream (5′) and downstream (3′) fragments (Fig. 2, lanes 2 and 6). Similarly, the *pap1* extract showed a normal cleavage activity at both temperatures (Fig. 2, lanes 5 and 9), because in contrast to the situation in higher eukaryotes (*1*), PAP is not required for cleavage (*2*, *4*). However, no cleavage product was detected when the *CYC1* precursor was incubated with *rna14* or *rna15* mutant extracts, whichever temperature was used (Fig. 2, lanes 3 and 4 and 7 and 8, respectively). We then mixed *rna14* and *rna15* extracts with the aim of obtaining a trans complementation of the inactivated components (*13*). Surprisingly, the *rna14* and *rna15* extracts consistently failed to complement one another (Fig. 2, lanes 10 and 13). This failure is presumably not due to an inhibitory component in the *rna14* or *rna15* extracts, because the extracts did not reduce the activity of the *pap1* extract when mixed with it (Fig. 2, lanes 11 and 12 or 14 and 15, respectively).

The polyadenylation reaction was then analyzed (*12*) with the *CYC1* precleaved precursor (Fig. 3, lane 1), which terminates at the natural polyadenylation site of the *CYC1* gene (*5*). In a wild-type extract, at either temperature, the *CYC1* precleaved RNA was specifically polyadenylated to give a species migrating as a broad band corresponding to the addition of 60 to 70 adenosine residues to the precursor (Fig. 3, lanes 2 and 6). In the *rna14* and *rna15* mutant extracts, polyadenylation of the precleaved precursor was strongly inhibited at both temperatures (Fig. 3, lanes 3 and 4 and 7 and 8, respectively), resulting in the formation of short poly(A) tails. This failure did not depend on the RNA used, because the *GAL7-6* precleaved precursor (*4*) could not be polyadenylated (*14*, *15*). In contrast to *rna14* and *rna15*, the temperature-sensitive mutant *pap1-5* produced poly(A) tails of normal length at 23°C, which completely disappeared at 32°C (Fig. 3, lanes 5 and 9, respectively) (*9*). As with the cleavage reaction (Fig. 2), the *rna14* and *rna15* extracts did not complement one another for polyadenylation (Fig. 3, lanes 10 and 13). However, combination of *rna14* or *rna15* extracts with the *pap1* extract resulted in a higher polyadenylation activity at 23° or 32°C than that shown by any of the mutant extracts alone (Fig. 3, lanes 11 and 12 and 14 and 15, respectively). Because *rna14* and *rna15* extracts can restore the polyadenylation activity of the *pap1* extract and vice versa, the failure to complement one another suggests that the RNA14 and RNA15 proteins may be tightly associated in a complex of nondissociable components required for cleavage and polyadenylation of yeast mRNA precursors.

We also analyzed the functions of RNA14 and RNA15 proteins by immunodepleting a wild-type extract with antibodies against these polypeptides (*16*, *17*). As above, the cleavage and polyadenylation activities were separately assayed with *CYC1* and the *CYC1* precleaved precursors, respectively. Pretreatment of the extract with antibody to either RNA14 or RNA15 almost completely inhibited both cleavage

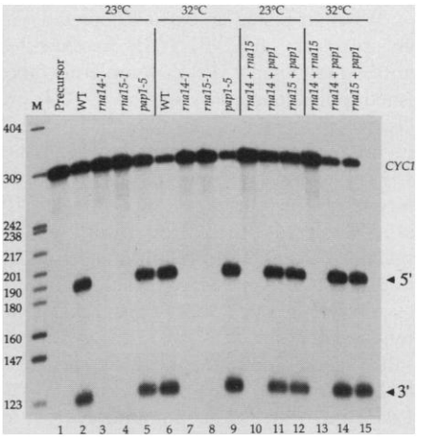

Fig. 2. Cleavage of the *CYC1* precursor RNA in vitro. The cleavage activity of the wild-type (WT, lanes 2 and 6), *rna14-1* (lanes 3 and 7), *rna15-1* (lanes 4 and 8), and *pap1-5* (lanes 5 and 9) extracts, and combinations of them (lanes 10 to 15), were assayed under standard conditions (*12*), except that cytidine triphosphate (CTP) replaced ATP, and EDTA was used instead of magnesium acetate. The reactions proceeded for 60 min at 23° or 32°C, as indicated. Precursor (lane 1), unreacted *CYC1* RNA (*24*); 5′ and 3′, upstream and downstream cleavage products, respectively. The labeled molecular weight markers (M) are pBR322 DNA digested with Hpa II and the sizes are indicated in number of nucleotides.

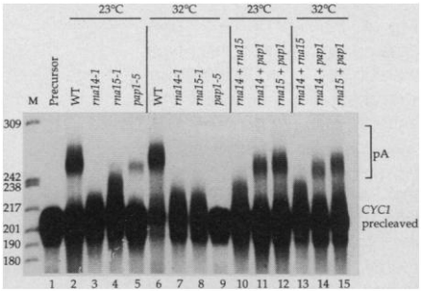

Fig. 3. Polyadenylation of the *CYC1* precleaved precursor in vitro (*12*). The *CYC1* precleaved transcript, ending at its natural polyadenylation site (*5*), was incubated at 23° or 32°C for 60 min in extracts made from the wild-type strain W303 (WT, lanes 2 and 6) and from mutant strains (*11*) carrying *rna14-1* (lanes 3 and 7), *rna15-1* (lanes 4 and 8), or *pap1-5* mutations (lanes 5 and 9), or combinations of them (lanes 10 to 15). Precursor (lane 1), unreacted *CYC1* precleaved RNA; pA, polyadenylated species; M, molecular weight markers indicated in number of nucleotides.

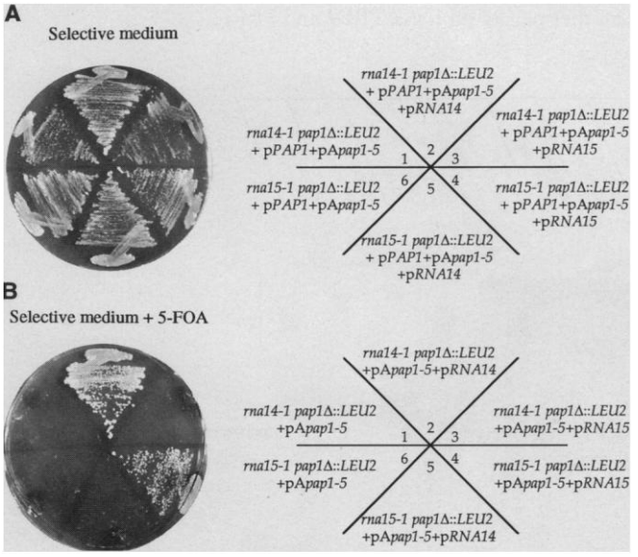

Fig. 1. Synergistic lethal interactions between *rna14* and *pap1* or *rna15* and *pap1* mutations. (**A**) Yeast strains containing either the *rna14-1* or the *rna15-1* temperature-sensitive alleles and a chromosomal disruption of the *PAP1* gene, rescued by the p*PAP1* plasmid (strains LM61 and LM62, respectively) (*8*), were transformed with the low-copy plasmid pA*pap1-5* carrying the temperature-sensitive allele *pap1-5* (*9*) (sectors 1 and 6); with pA*pap1-5* and the low-copy plasmid p*RNA14* bearing the *RNA14* wild-type gene (sectors 2 and 5, respectively); or with pA*pap1-5* and the low-copy plasmid p*RNA15* bearing the *RNA15* wild-type gene (sectors 3 and 4, respectively). Equal numbers of cells of these transformants were streaked at 24°C on selective medium as sectors. (**B**) The same transformants as in (A) were plated on a selective medium containing 5-FOA, on which the *URA3*-based p*PAP1* plasmid is lost (*10*). The genotypes of the transformants are depicted on the right.

and polyadenylation activities (Fig. 4, lanes 1, 3, 9, and 11, respectively), whereas the corresponding preimmune sera had no significant effect (Fig. 4, lanes 2, 4, 10, and 12, respectively), in comparison with the untreated extract (Fig. 4, lanes 8 and 16). This experiment reproduced the results obtained with the mutant extracts (see Figs. 2 and 3), strengthening the genetic prediction that a component required for 3′-end processing is specifically inactivated by mutations in *RNA14* or *RNA15* genes.

We next tested an extract depleted with an antiserum to PAP1 (*18*). As expected, antibodies against PAP completely abolished polyadenylation of the *CYC1* precleaved precursor (Fig. 4, lane 13), whereas preimmune serum had no effect (Fig. 4, lane 14). However, we observed a drastic inhibition of the cleavage reaction with the same depleted extract; this inhibition was specific because the preimmune serum did not affect cleavage (Fig. 4, lanes 5 and 6, respectively). Because in yeast PAP is not required for the cleavage reaction, this result suggests that the cleavage factor or factors are associated in a complex with PAP and can be coimmunoprecipitated with antibodies to PAP1.

To elucidate the role of the RNA14 and RNA15 proteins in 3′-end processing, we studied four functionally distinct factors that are required for 3′-end processing in vitro (*4*). We separated these factors (PAP, CF I, CF II, and PF I) on a Mono Q column (*4*) and showed that the combination of the *rna14* extract with fractions 34 to 46, where the so-called CF I activity elutes, perfectly reconstituted the maturation of the *CYC1* pre-mRNA (Fig. 5A, lanes 5 and 11) (*19*). This result was expected, because CF I is the only component required not only for cleavage but for polyadenylation as well (*4*). The residual cleavage activity observed in the later eluting fractions (48 and 50) (Fig. 5A, lanes 12 and 13, respectively) was due to an overlap of CF I– with CF II–containing fractions, the latter eluting at higher salt concentrations (*4*).

To demonstrate that RNA14 and RNA15 proteins correlate with CF I function, we did a protein immunoblot analysis across the Mono Q fractions that were used to complement *rna14* and *rna15* deficiency. Antisera to RNA14 and RNA15 detected polypeptides corresponding in size to RNA14 (80 kD) and RNA15 (42 kD) proteins, respectively (*16*). These polypeptides were in the same fractions that complemented the mutant extract deficiency (Fig. 5B, fractions 34 to 46). [The band below RNA14 protein is not related to RNA14, because it was also detected with the corresponding preimmune serum (*14*).]

The use of genetic and biochemical methods allowed the identification of RNA14 and RNA15 proteins as essential components of CF I involved in both cleavage and polyadenylation of mRNA precursors in yeast. The nuclear localization of RNA14 and RNA15 proteins is consistent with this role (*16*). Our results biochemically confirm earlier genetic evidence (*6*) suggesting that the proteins may act in the same complex. Furthermore, the fact that the *rna14* and *rna15* mutant extracts do not complement each other even though they separately complement a *pap1* extract suggests that they are part of a tight complex, whose components cannot exchange (under the reaction conditions tested). The same kind of result has been reported for another pair of proteins, PRP9 and PRP11, which interact with each other and with the U2 snRNP in the yeast spliceosome (*13*).

Because *RNA14*, *RNA15*, and *PAP1* are single-copy genes in the yeast genome, the synergistic lethality observed in the different combinations of double mutations reported here and elsewhere (*6*) makes it very likely that their products have no redundant functions in 3′-end processing. It has been suggested that CF I, which is the only factor required for both cleavage and polyadenylation, may contain a component that binds to the cis-acting sequences upstream of the poly(A) site in pre-mRNAs (*4*). RNA15 protein is an excellent candidate for that function, as it contains a canonical RNA-binding domain (*6*). Moreover, in vitro binding experiments have shown that

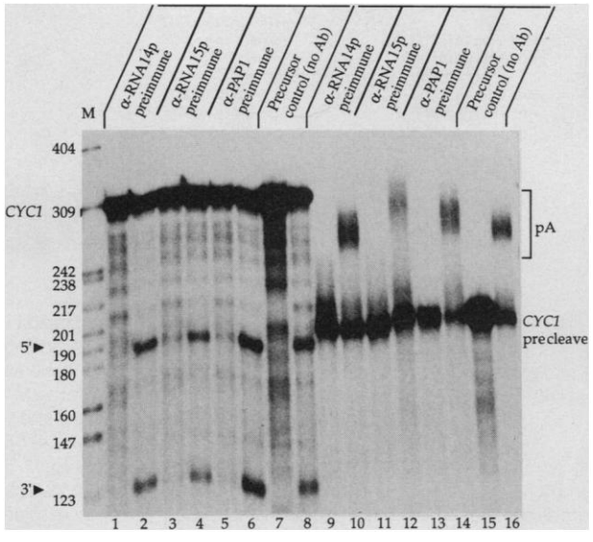

Fig. 4. Inactivation of 3′ processing activity of a wild-type extract by immunodepletion with antibodies (*17*). A wild-type extract was immunodepleted with antibodies to RNA14 protein (α-RNA14p), to RNA15 protein (α-RNA15p), to PAP (α-PAP1), or with their corresponding preimmune sera (preimmune). The cleavage (lanes 1 to 8) and polyadenylation (lanes 9 to 16) reactions were analyzed separately, as described in Figs. 2 and 3. The incubation was done at 30°C for 30 min. Precursor control (no Ab): activity of the wild-type extract not subjected to antibody depletion. 5′, 3′, and pA are the reaction products (as defined in Figs. 2 and 3). Molecular weight markers (M) are indicated in number of nucleotides.

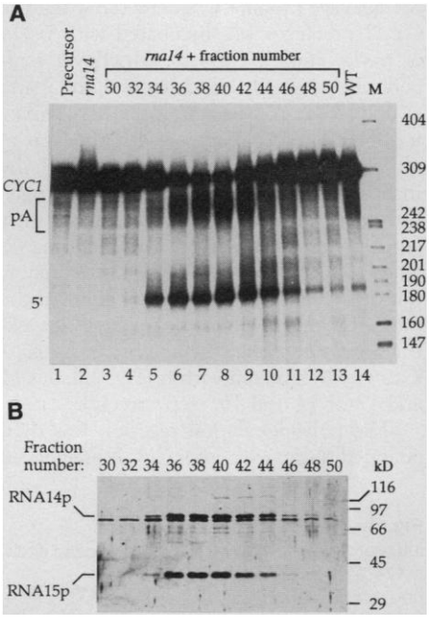

Fig. 5. Reconstitution of 3′-end formation in *rna14* extract by complementation with partially purified CF I fractions containing RNA14 and RNA15 polypeptides. (**A**) 3′ processing activity of an *rna14* extract is restored by mixing 6 μl of the Mono Q fractions [obtained as described in (*4*)] with 2 μl of *rna14* extract under standard reaction conditions (*13*). The full complementing activity (cleavage and polyadenylation of the *CYC1* precursor) correlates with fractions 34 to 46 (lanes 5 to 11). The reactions were done at 30°C for 30 min. M, molecular weight markers (in number of nucleotides). (**B**) Antibodies to RNA14p and RNA15p revealed RNA14 and RNA15 proteins in the complementing CF I fractions. Four microliters (approximately 2.2 μg of proteins) of Mono Q fractions 30 to 50 were subjected to a protein immunoblot analysis according to standard procedures (*25*). The same blot was probed simultaneously with antisera to RNA14p (diluted 1:2500) and to RNA15p (at a 1:1000 dilution). The molecular mass and migration position of protein markers are indicated on the right.

RNA15 can bind to poly(U) ribopolymers (*20*), which suggests that the generally U-rich RNA sequences important for 3′ processing in yeast (*4*, *21*) may be recognized by this protein. The finding that this putative RNA-binding protein is a component of CF I may thus help to elucidate the sequence requirements in yeast 3′-end processing. It is worth noting in this context that the mammalian counterpart of yeast CF I, cleavage and polyadenylation specificity factor (CPSF), is a sequence-specific RNA-binding factor consisting of multiple polypeptides (*1*, *22*).

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8. The relevant genotypes of the starting strains are as follows: LM61 (*rna14-1*, *pap1*Δ*::LEU2*, *ura3-1*, *trp1-1*, *ade2-1*, and p*PAP1*) and LM62 (*rna15-1*, *pap1*Δ*::LEU2*, *ura3-1*, *trp1-1*, *ade2-1*, and p*PAP1*). p*RNA14* and p*RNA15* are *TRP1*-marked low-copy plasmids containing genomic fragments allowing complementation of *rna14* and *rna15* mutations, respectively. pA*pap1-5* is a low-copy *ADE2*-marked plasmid carrying the *pap1-5* mutant allele (*9*, *23*).
9. P. J. Preker and W. Keller, unpublished results. Eight different temperature-sensitive mutant alleles, called *pap1-2* to *pap1-9*, were generated by polymerase chain reaction mutagenesis with the use of either low deoxyadenosine triphosphate (dATP) concentration or inclusion of manganese in the reaction. These mutants were sequenced, and most of them showed multiple mutations. In vitro, the extract of the *pap1-5* mutant used in this study shows approximately 50% of specific polyadenylation activity as compared with that of a wild-type extract.
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11. The extracts were made as described elsewhere (*2*), except that cells were grown at 24°C and converted to spheroplasts with the use of Zymolyase-100T (Seikagaku Kogyo, Tokyo) at a concentration of 300 μg/ml. The names and genotypes of the strains are as follows: LM88 (*rna14-1*; *ura3-1*; *trp1-1*; *ade2-1*; *leu2-3,112*), LM91 (*rna15-1*; *ura3-1*; *trp1-1*; *ade2-1*; *leu2-3,112*), and LM98 (*ura3-1*; *trp1-1*; *ade2-1*; *leu2-3,112*; *his3-11,15*; *pap1*Δ*::LEU2*; and pA*pap1-5*). They are isogenic with strain W303 (*ura3-1*; *trp1-1*; *ade2-1*; *leu2-3,112*; *his3-11,15*, from R. Rothstein, Columbia University, New York), which was used to prepare the wild-type extract. The pA*pap1-5* plasmid contains the *pap1-5* mutant allele (*9*) cloned into an *ADE2*-marked low-copy vector (pASZ11) (*23*).
12. A standard in vitro processing reaction was done in a 25-μl reaction volume containing 2 μl of extract, 1.6 mM Hepes-KOH (pH 7.9), 0.016 mM EDTA, 4 mM potassium chloride, 1.04 mM dithiothreitol, 1.6% glycerol, 2% polyethylene glycol, 75 mM potassium acetate, 1.8 mM magnesium acetate, 2 mM ATP, 20 mM creatine phosphate, creatine kinase (0.2 mg/ml), 0.01% NP-40, and ~0.2 units of RNAguard (Pharmacia). When only the cleavage reaction was assayed, CTP replaced ATP, and EDTA was used instead of magnesium acetate, which prevents poly(A) addition and degradation of the 3′ fragment (*4*). The reactions were incubated at the temperatures and for the times indicated in the figure legends. They were stopped by addition of 75 μl of a stop solution [100 mM tris-HCl (pH 8), 150 mM NaCl, 12.5 mM EDTA, 1% SDS, proteinase K (0.2 mg/ml), and glycogen (0.05 mg/ml)] and incubated for 1 hour at 42°C. The RNAs were recovered by precipitation and analyzed as described (*4*). For complementation assays, the extracts were mixed in a 1:1 ratio.
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14. L. Minvielle-Sebastia, unpublished results.
15. The synthesis of the short tracts observed with *rna14* and *rna15* mutant extracts still depends on the $(UA)_6$ cis-acting sequences of the *GAL7* precursor, because no polyadenylation was obtained with the mutant precleaved RNA (*GAL7-7*), in which these signals were deleted (*4*).
16. N. Bonneaud, L. Minvielle-Sebastia, C. Cullin, F. Lacroute, *J. Cell Sci.* **107**, 913 (1994).
17. Fifty microliters of crude anti-serum or preimmune serum directed against RNA14p, RNA15p, or PAP1 was coupled to ≈40 μl of packed protein A–Sepharose (PAS) preequilibrated with buffer E (*4*) plus 0.01% NP-40 for 3 hours at 4°C. After three washes with the same buffer, 70 μl of a wild-type extract was added to the resin and incubated for 4 hours at 4°C on a wheel. The supernatant was reapplied on a fresh antibody-PAS resin for a second round of depletion. The wild-type strain used here and for the fractionation of the 3′-processing factors is a commercial brewery *S. cerevisiae* strain, referred to as VDH2 (Versuchanstalt der Hefeindustrie, Berlin, Germany). The cells were broken in a Bead Beater (BioSpec, Bartlesville, OK), and the protein extract was further prepared as previously described (*4*). Extracts made from this strain were active for 3′ processing of *CYC1*, *GAL7*, and their corresponding precleaved RNAs (*14*).
18. J. Lingner and W. Keller, unpublished results. The serum was obtained from a rabbit immunized with recombinant PAP (*5*).
19. An identical pattern of complementation was also found with the *rna15* extract. In the same way, CF I fraction 40 restored 3′-end processing activity of the extracts depleted by antibodies to RNA14 and RNA15 (*14*).
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Correction of Lethal Intestinal Defect in a Mouse Model of Cystic Fibrosis by Human *CFTR*

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Cystic fibrosis (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (*CFTR*). A potential animal model of CF, the *CFTR*$^{-/-}$ mouse, has had limited utility because most mice die from intestinal obstruction during the first month of life. Human *CFTR* (h*CFTR*) was expressed in *CFTR*$^{-/-}$ mice under the control of the rat intestinal fatty acid–binding protein gene promoter. The mice survived and showed functional correction of ileal goblet cell and crypt cell hyperplasia and cyclic adenosine monophosphate–stimulated chloride secretion. These results support the concept that transfer of the h*CFTR* gene may be a useful strategy for correcting physiologic defects in patients with CF.

Cystic fibrosis mice bearing a null mutation in the *CFTR* gene lack adenosine 3′,5′-monophosphate (cAMP)–stimulated Cl^- transport in intestinal epithelial cells, which leads to goblet cell hyperplasia, intestinal obstruction, and perforation (*1*). To correct the lethal intestinal abnormalities in a group of CF mice, we used the rat intestinal fatty acid–binding protein (FABP) gene promoter (*2*) to direct expression of the wild-type h*CFTR* complementary DNA (cDNA) to the intestinal epithelial cells of these mice (*3*). A chimeric FABP-h*CFTR* gene construct was microinjected into fertilized oocytes, producing

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