Proc. Natl. Acad. Sci. USA
Vol. 77, No. 4, pp. 2119–2123, April 1980
Genetics

Isolation of genes by complementation in yeast: Molecular cloning of a cell-cycle gene

(*Saccharomyces cerevisiae*/*cdc28*/recombinant plasmid/transformation/plasmid integration)

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Communicated by Herschel L. Roman, November 29, 1979

ABSTRACT ***cdc28*, one of several genes required for cell division in the yeast *Saccharomyces cerevisiae*, has been isolated on recombinant plasmids. A recombinant plasmid pool containing the entire yeast genome was constructed by partial digestion of yeast DNA with the four-base recognition restriction endonuclease *Sau*3A to give the equivalent of random fragments, size selection on sucrose gradients, and introduction of the fragments into the yeast vector YRp7 by use of the homology of *Sau*3A ends with those generated in the vector by cleavage with *Bam*HI. Recombinant plasmids capable of complementing *cdc28* mutations were isolated by transformation of a $cdc28^{ts}$ strain and selection for clones capable of growth at the restrictive temperature. Plasmids responsible for complementing the $cdc28^{ts}$ phenotype were shown to recombine specifically with the chromosomal *cdc28* locus, confirming the identity of the cloned sequences. In addition, one of the recombinant plasmids was capable of complementing a mutation in *tyr1*, a gene genetically linked to *cdc28*. This method of gene isolation and identification should be applicable to all yeast genes for which there are readily scorable mutants.**

Recent advances in recombinant DNA methodology, in particular the discovery of a means to introduce DNA directly and stably into yeast cells (transformation) (1, 2), suggest a possible route to the identification of hitherto uncharacterizable gene products in this organism. Genes may first be selected from a plasmid pool by requiring that they complement a specific mutation when introduced by transformation. Complementary mRNA can then be obtained and translated *in vitro* to identify gene products.

cdc28 is one of several genes required for cell division in yeast (*Saccharomyces cerevisiae*) (3–5), whose gene product has not been identified. We report here the molecular cloning of the *cdc28* gene as the first step of a program aimed at identifying and characterizing the *cdc28* protein. The method used should be applicable to any yeast gene for which there are readily scorable mutants. From a pool of wild-type yeast DNA fragments carried in the plasmid vector YRp7 (6), we were able to select several hybrid plasmids containing *cdc28* sequences on the basis of their ability to complement a temperature-sensitive *cdc28* mutation in transformation experiments. The identity of these clones was confirmed by their ability to recombine specifically with homologous sequences at the chromosomal *cdc28* locus in transformant yeast cells and by the discovery that one of them also contained sequences capable of complementing mutations in a linked gene, *tyr1* (refs. 4 and 7 and unpublished results).

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MATERIALS AND METHODS

Organisms, DNAs, Enzymes, and Media. The following strains were used: yeast, SR 668-2 (*mat α, tyr1, leu2, trp1, ade1, cyh2, cdc28-4*) and KN 86 (*mat a, cdc28-4, trp1, lys2, his7*); *Escherichia coli*, RRI (F^- *pro leu thi lacy* Str^r r_K^- m_K^- $endoI^-$) and *trpC* 9830 containing plasmid YRp7 (6). Plasmid DNA was prepared by the methods of Bolivar *et al.* (8) and Elwell *et al.* (9). Yeast DNA prepared from AB320 (HO, *ade2-1, lys2-1, trp5-2, leu2-1, can1-100, ura3-1,* and/or *ura1-1, met4-1*) was a gift from M. Olson. AB320 is a segregant from W87 (10). All restriction enzymes, T4 DNA ligase, and bovine serum albumin were purchased from New England Biolabs and Bethesda Research Laboratories (Rockville, MD) and used as directed, except that bovine serum albumin was always included at 100 μg/ml. All media used for the culture of yeast cells have been described (11).

Construction of a Pool of Yeast DNA Sequences in YRp7. Unless otherwise stated, all cloning procedures involving *E. coli* were as described by Goodman *et al.* (12). Yeast DNA from AB320 was partially cleaved with three different concentrations of *Sau*3A (0.5, 1.0, and 2.0 relative units) so that its average size was approximately 10 kilobases (kb). It was then pooled and separated according to size on sucrose gradients as described by Maniatis *et al.* (13), and fragments between 5 and 20 kb were ligated with YRp7 DNA that had been digested with *Bam*HI and bacterial alkaline phosphatase (the respective DNA concentrations were 50 and 10 μg/ml). After ligation, the DNA was made 20 mM in EDTA (pH 8.0) and used to transform *E. coli* strain RRI to ampicillin resistance (Amp^R); 0.1 ml of ligation mixture produced 8.2×10^4 Amp^R colonies, of which half were tetracycline sensitive (Tet^S). Plasmid DNA was prepared from a random 10 of the latter, and all were shown to contain a yeast DNA insert by an analysis of their restriction patterns with *Eco*RI. The average size of this sample of inserts was ≥7.5 kb. The transformant colonies were scraped from the ampicillin plates and the cells were pooled and pelleted by centrifugation. Half were stored in several aliquots as described by Beggs (2) at −70°C and the other half in 10 mM $MgCl_2$/50% (vol/vol) glycerol at −20°C.

Small-Scale Preparation of Yeast DNA. Five milliliters of stationary phase culture in YEP medium or 10 ml of culture in minimal medium was harvested by centrifugation, washed with 1 ml of distilled H_2O, and suspended in 0.2 ml of 1.0 M sorbitol, 0.1 M sodium citrate (pH 7.0), 60 mM EDTA, 1 mg of zymolyase-5000 per ml (Kirin Brewery, Japan), and 1% 2-mercaptoethanol. After incubation at 37°C for 30 min, 0.8 ml of 0.5%

Abbreviations: kb, kilobases; Amp^R, ampicillin resistance.

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NaDodSO4/100 mM Tris·HCl, pH 9.7/50 mM EDTA, pH 8.5, was added and the sample was mixed well by inversion. The mixture was then heated at 70°C for 20 min and cooled; 0.2 ml of 5 M potassium acetate was added and the mixture was left at 0°C for 45 min. After centrifugation in a microfuge for 5 min at 4°C, the supernatant was precipitated with 2 vol of ethanol (20 min at −70°C) and the pellet obtained by centrifugation was dissolved in 10 mM Tris·HCl, pH 8.0/1 mM EDTA, made 0.25 M in NaCl, and reprecipitated with ethanol; the pellet was dissolved in 50 μl of Tris/EDTA. This preparation contained both chromosomal and plasmid DNA species and was used for gel electrophoresis and *E. coli* transformation.

Yeast Transformation. The procedure described by Beggs (2) was used with minor modification. At low DNA concentrations (<5 μg/ml), at which the yield of transformants is less than expected, pBR322 DNA (8) was added at 10 μg/ml. This increased the yield of transformants between 10- and 100-fold.

Nucleic Acid Hybridization. ^{32}P-Labeled probes were prepared and hybridized to DNA transferred to nitrocellulose filters as described by Montgomery *et al.* (14), with the modification that the liquid-to-filter ratio was 0.09 ml/cm^2, sheared calf thymus DNA was added to 4 μg/cm^2, and the hybridization proceeded while the sample was rocked in a sealed plastic bag at 65°C.

RESULTS

Construction of a Pool of Yeast DNA Sequences in YRp7. The vector chosen for the construction of a pool of yeast sequences was the hybrid plasmid YRp7 (6). YRp7 is composed of a 1.4-kb yeast *Eco*RI fragment containing the *TRP1* gene inserted into the *Eco*RI site of bacterial plasmid pBR322. In addition to providing *TRP1* function (anthranilate isomerase activity), this yeast fragment enables the molecule to replicate autonomously in yeast as a low-copy-number, closed circular molecule (6).

A pool of yeast DNA sequences was constructed in YRp7 by a modification of the procedure of Maniatis *et al.* (13). Yeast DNA was partially digested with the four-base recognition site enzyme *Sau*3A and fragments 5–20 kb long were isolated by centrifugation in sucrose gradients. Because *Sau*3A leaves the same G-A-T-C cohesive end as does the six-base recognition site enzyme *Bam*HI, these fragments can be inserted by ligation directly into the *Bam*HI site of YRp7. The pool so constructed was stored and amplified by transformation of *E. coli* to ampicillin resistance. It contained at least 2.5 × 10^4 recombinant clones; each yeast gene should, therefore, be represented in whole or part on about 10 plasmids of independent origin.

The procedure of ligating *Sau*3A to *Bam*HI cohesive ends dispenses with the necessity of attaching synthetic linkers [whether dA-dT tails or synthetic restriction sites (13)] in order to create a pool composed of random or quasirandom fragments.

Transformation of a *cdc28* Mutant with DNA from the Pool. When used to transform a *trp1*$^-$ *cdc28*^{ts} strain (SR 668-2), DNA prepared from the yeast YRp7 hybrid pool yielded 2 × 10^4 TRP$^+$ transformants per μg (representing 0.06% of the cells capable of regeneration after being made into spheroplasts) of which 0.04% were capable of growth at the restrictive temperature (38°C). A total of 50 TRP$^+$ CDC$^+$ transformants were isolated. DNA from several of these was analyzed by gel electrophoresis without prior digestion by use of a pBR322 ^{32}P-labeled probe to detect plasmid sequences. Fig. 1 shows that the transformants possessed a variety of different forms containing pBR322 sequences. High molecular weight linear DNA forms, suggestive of chromosomal integration, as well as autonomous plasmid forms were observed. Frequently, both forms coexisted in the same transformant, as did, in some cases, plasmids of several different sizes.

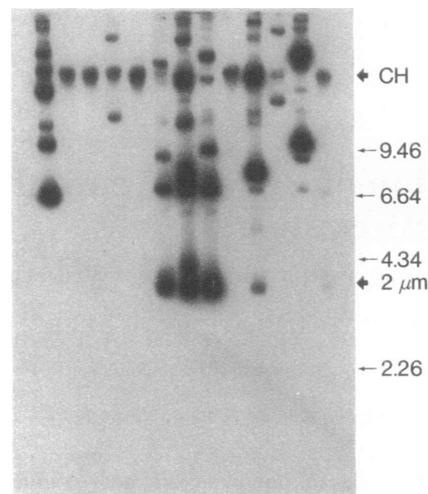

FIG. 1. Detection of pBR322 sequences in *TRP*$^+$ *CDC*$^+$ transformants. DNA was prepared from SR688-2 and several transformants, electrophoresed in a 0.5% agarose gel for 17 hr at 1 V/cm, transferred to a nitrocellose filter by the method of Southern (15), and hybridized to 8 × 10^6 cpm of ^{32}P-labeled pBR322 DNA. The positions in the gel of the chromosomal DNA band (CH), closed circular 2-μm DNA monomers (2 μm), and molecular weight markers (in kb) were detected prior to transfer by staining in ethidium bromide. From left to right: transformant 3.93.2, 3.96.19, .8, .12, .9, .26, .28, .20, .23, .13, .29, .25, .27, and SR688-2.

Isolation of Putative *cdc28*-Containing Plasmids. Most of the transformants rapidly lost both TRP$^+$ and CDC$^+$ phenotypes when grown under permissive conditions (data not shown). This instability is characteristic of YRp7 (6) and suggests that the gene responsible for complementing *cdc28* in such transformants is carried on a plasmid. In order to isolate and amplify such plasmids, we introduced DNA from the transformants into *E. coli* by transformation to ampicillin resistance, with a yield between 0 and 10^4 Amp^R clones per μg, depending upon its source. Rapid preparations of plasmid DNA were made from Amp^R Tet^S clones and analyzed by digestion with restriction endonucleases followed by agarose gel electrophoresis. Three plasmids, each isolated from a different yeast transformant and containing a different sized insert, shared extensive DNA sequence homology based on restriction endonuclease digestion patterns: YRp7·*CDC28*(1), (2), and (3). (All contained a characteristic set of *Hin*dIII fragments; see Fig. 2.) Due to this homology, these plasmids were considered likely to contain the sequences responsible for complementation of the *cdc28* mutation.

Cloned Sequences Were Capable of High-Efficiency Transformation of *cdc28*^{ts} Mutants. Table 1 presents data obtained when plasmids YRp7·*CDC28*(2) and (3) were reintroduced into the *cdc28*^{ts} strain SR688-2 by transformation. In each case, 2–3 × 10^4 colonies capable of growth at the restrictive temperature (selection for CDC$^+$ phenotype only) were observed per μg of transforming DNA. Similar results were obtained when selecting instead for tryptophan prototrophy (TRP$^+$) at the permissive temperature (23°C).

All TRP$^+$ colonies selected at the permissive temperature were capable of growth at the restrictive temperature. However, such colonies required a significantly longer interval to appear than those produced by the vector YRp7, suggesting that the expression of *TRP1* can be affected by inserted DNA sequences. YRp7·*CDC28*(1) was also capable of complementing

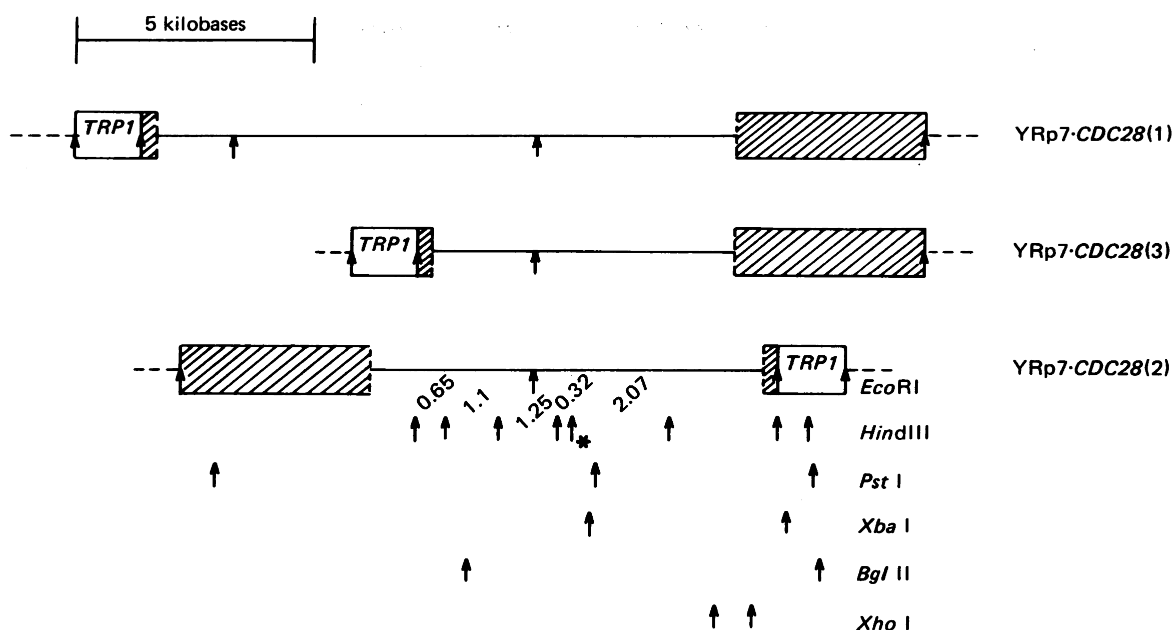

FIG. 2. Restriction map of YRp7·*CDC28*(1), (2), and (3). The three plasmids are drawn so that their overlapping inserts are aligned. The boxed sections represent vector sequences. pBR322 sequences are hatched. The position of *Eco*RI sites is shown for all plasmids. The positions of other sites are shown for YRp7·*CDC28*(2). The sizes of the various restriction fragments were estimated from gel electrophoresis (0.7 and 1.5% agarose). Fusion fragments between vector and insert were detected by hybridization to ^{32}P-labeled pBR322 by the method of Southern (15). The fusion fragments created by most of the enzymes contained either a large (4 kb), strongly hybridizing or small (0.3 kb), weakly hybridizing tract of pBR322 sequence. As a result, the orientation of the insert and the location of at least two sites internal to it could be unambiguously assigned from an analysis of limit fragments produced by single digestions. This method showed the location of all *Eco*RI, *Pst* I, *Xba* I, *Bgl* II, and *Xho* I sites. There was no *Bam*HI site in YRp7·*CDC28*(2) and (3). The position of the more numerous *Hin*dIII sites was partly assigned by double digestions (e.g., *Hin*dIII/*Eco*RI, *Xba* I, *Bgl* II, or *Xho* I) and partly by their topology in other plasmids (not reported here). For example, one contained only the 2.07-, 0.32-, and 1.25-kb fragments and another only the 2.07- and 0.32-kb fragments. The asterisk marks the uncertainty concerning the exact position of one of the *Hin*dIII sites.

both *trp1* and *cdc28*^{ts} mutations (data not shown). In addition, it alone was capable of complementing the *tyr1* mutation present in SR668-2.

Plasmids YRp7·*CDC28*(2) and (3) Contain Sequences Capable of Recombination at Chromosomal *cdc28* Locus in Transformant Cells. Complementation of mutations is not sufficient proof that cloned sequences contain the gene being sought. However, the fact that hybrid plasmids derived from the vector YRp7 were capable of stable chromosomal integration (see legend to Table 2), presumably by mitotic recombination with homologous chromosomal sequences, allowed a more stringent test of identity. In Table 2, we present genetic evidence for the stable integration of plasmids YRp7·*CDC28*(2) and (3) at the *cdc28* locus, confirming that these plasmids do, in fact, contain the *cdc28* gene. The position of the integrated sequences within the yeast genome was determined by tetrad analysis. The experiment was designed so that *tyr1*, a locus closely linked to *cdc28* on chromosome II (7 meiotic map units), established the normal chromosomal *cdc28* position (ref. 7 and unpublished data). The *TRP1* allele associated with the vector moeity of the plasmid marks the point of integration. Thus, if *trp1* and *tyr1* show strong genetic linkage, the position of the integrated plasmid is established as at or near the *cdc28* locus. The chromosomal *trp1* locus is near the centromere of chromosome IV, but because both alleles at this position are mutant, the locus is silent in this experiment. For each of two independently observed integration events of plasmids YRp7·*CDC28*(2) and (3), the calculated map distance between *tyr1* and *trp1* was

Table 1. Transformation of SR668-2 with purified plasmids

Plasmid	Complete medium,* 38°C	Tryptophanless medium 23°C	Tryptophanless medium 38°C
YRp7·*CDC28*(2)	3.2×10^4, 3.1×10^4	3.3×10^4	0.4×10^4
YRp7·*CDC28*(3)	2.7×10^4, 2.1×10^4	1.3×10^4	0.3×10^4
YRp7	—	7.0×10^4	—

Transformations are reported as transformant colonies per μg of transforming DNA.
* Values reported from each of two independent experiments.

Table 2. Genetic linkage of integrated plasmids to *tyr1*

Integrant strain	Ascus type PD	NPD	T	Calculated map distance
YRp7·*CDC28*(2)-int 1	11	0	2	8 ± 5
YRp7·*CDC28*(2)-int 2	16	0	1	3 ± 4
YRp7·*CDC28*(3)-int 1	16	0	1	3 ± 4
YRp7·*CDC28*(3)-int 2	12	0	3	10 ± 5

Strains containing integrated copies of plasmid were first constructed by transforming SR668-2 (*cdc28-4, mat α, trp1, leu2, ade1, tyr1*) with the appropriate purified plasmid and by repeatedly streaking on solid medium without tryptophan at 38°C. Large colonies, which eventually appeared, were assumed to have resulted from an integration event, and these were tested for stability of the plasmid-associated phenotype. Such stable transformant strains were referred to by the plasmid designation followed by a numerical identification; for example: YRp7·*CDC28*(2)-int 1. For mapping, these strains were crossed to KN-86 (*cdc28-4, mat a, trp1, lys2, his7*). Tetrad analysis was carried out as described (16). In all cases, map distance reported is that between chromosomal *tyr1* and plasmid-associated *trp1*. Map distance is expressed in meiotic map units, where 1 map unit represents the equivalent of one crossing-over event per 50 meioses (16). *cdc28* and *tyr1* are normally separated by 7 map units (ref. 7 and unpublished data). Error limits given are ±SD.

between 3 and 10 meiotic map units, which is consistent with the *cdc28-tyr1* map distance within the limits of statistical expectation based on the sample size. The same result was obtained if one considered the linkage between *cdc28* and *tyr1*.

Structure of *cdc28*-Containing Plasmids Was Established by Restriction Endonuclease Mapping and Heteroduplex Analysis. Fig. 2 shows that YRp7·*CDC28*(1), (2), and (3) contain 12.25, 6.4, and 8.3 kb of inserted yeast DNA, respectively. The insert of YRp7·*CDC28*(2) has an orientation opposite that of YRp7·*CDC28*(1) and (3). Because only YRp7·*CDC28*(1) is capable of complementing *tyr1*, the left-hand part of the diagram is presumed to be distal to the centromere of chromosome II.

Heteroduplex analysis of plasmids YRp7·*CDC28*(1), (2), and (3) confirmed the existence of DNA homology between the various yeast sequences as well as their relative orientation within the vector (see Fig. 3). If heteroduplex molecules were prepared between *Xho* I-cleaved plasmids YRp7·*CDC28*(2) and (3), two classes of molecule predominated (Fig. 3*A*). These are anticipated if the yeast DNA sequences within these plasmids span a similar genomic region but are placed in the vector in an opposite sense from one another. Heteroduplex molecules between *Xho* I-cleaved plasmids YRp7·*CDC28*(1) and (3) were of only one type, shown in Fig. 3*B*. The observed deletion loop is consistent with the inserted sequences of these two plasmids having parallel orientation and a single common end point near the *Xho* I-cleavage site. The deletion loop represents a region where no corresponding homology exists between plasmids YRp7·*CDC28*(1) and (3) due to the larger size of the yeast DNA insert of the former (12.25 kb as compared to 6.4 kb).

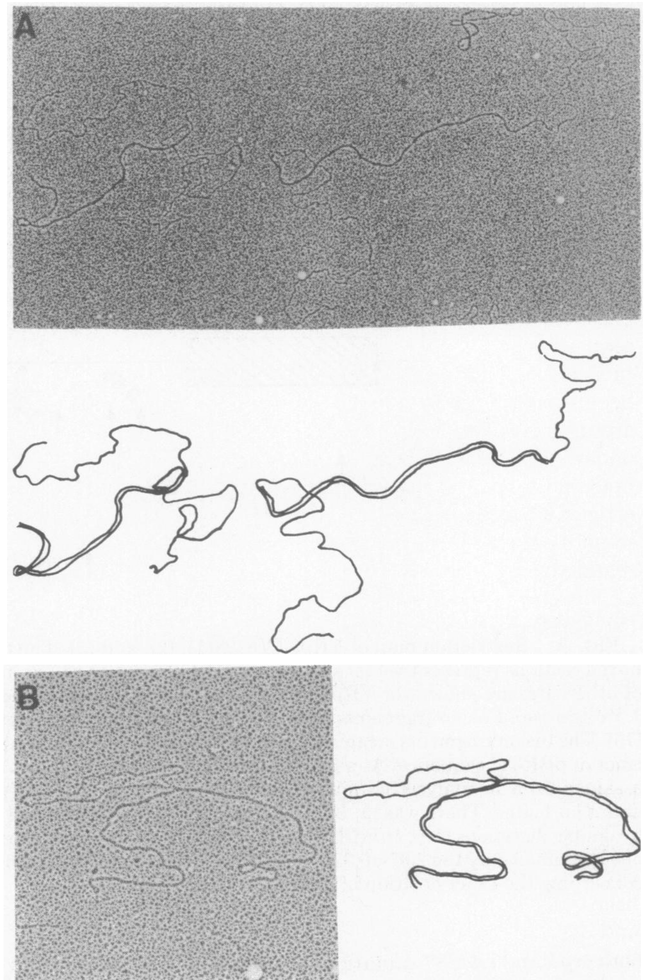

FIG. 3. Plasmids YRp7·*CDC28*(1), (2), and (3) were treated with endonuclease *Xho* I. Reaction mixtures were then extracted with 2 parts of a 1:1 mixture of phenol and chloroform. After removal of the aqueous phase, the organic phase was reextracted with an equivalent volume of Tris/EDTA and aqueous phases were then pooled, made 0.1 M in NaCl, and precipitated with 3 vol of absolute ethanol at −70°C for 1 hr. DNA was collected by centrifugation in an Eppendorf microfuge and pellets were air dried followed by resuspension in Tris/EDTA. Heteroduplex molecules were constructed and prepared for electron microscopic observation as described (17, 18). Electron microscopy and photomicrography were performed on a Phillips EM300 microscope equipped with a 4 × 4 inch plate camera. (*A*) Typical heteroduplex structures seen with DNA from plasmids YRp7·*CDC28*(2) and (3). (*B*) Typical heteroduplex molecule seen with DNA from plasmids YRp7·*CDC28*(1) and (3).

DISCUSSION

In their papers on the high-frequency transformation of yeast by autonomously replicating hybrid vectors, Beggs (2) and Struhl *et al.* (6) allude to the potential of using hybrid vectors for the *de novo* isolation of genes by complementation of yeast mutants. One of the main attractions of such a procedure lies in its potential for identifying the products of genes that are presently only characterized by the gross phenotype of mutants. We describe here the *de novo* isolation and identification of one such gene, *CDC28*, by means of its ability to complement a temperature-sensitive *cdc28* mutant. We believe that this method of gene isolation should be applicable to any yeast gene for which there are readily storable mutants.

The first step in the isolation of genes by complementation is the construction of a recombinant yeast DNA pool in a yeast vector. For the pool to be of general use, it is essential that it consist of random or quasirandom yeast DNA fragments. Pools constructed simply with DNA fragments resulting from limit restriction endonuclease digestion suffer from two limitations: a certain proportion of genes will contain a restriction site and will therefore exist as nonfunctional fragments and a particular gene will always be associated with a particular sized fragment and will therefore be selected for or against at the time of ligation (large fragments, for instance, will tend to be underrepresented). We have used a simplified method to give the equivalent of a randomly sheared pool with a minimum of enzymatic and physical manipulations. Partial digestion with the four-base recognizing endonuclease, *Sau*3A, followed by size fractionation on sucrose gradients has been substituted for physical shearing of the DNA to desired size. Randomness of fragment end point is approximated by the high frequency of *Sau*3A recognition sites predicted in yeast DNA (one site per 276 base pairs). Maniatis and coworkers (13) have reported a similar procedure for generating the equivalent of a randomly sheared pool by using DNA linker sequences in order to introduce the fragment pool into the cloning vector. Our technique uses the homology of the ends generated by *Sau*3A digestion with those generated by the six-nucleotide recognizing enzyme *Bam*HI, allowing direct introduction of the fragments into the *Bam*HI sites of several vector systems.

We have used the vector YRp7, which can be readily moved from a bacterial host to a yeast host and back again, all by direct transformation (6). Thus, the YRp7 plasmid pool can be transferred (in bulk) from *E. coli*, where it is stored and amplified, to *trp1*$^-$ yeast mutants, where the requirements of complementation may be imposed in order to isolate particular hybrid plasmids. This is performed by demanding simultaneous transformation of *trp1* and the mutant locus in question. A double selection such as this ensures that the level of transformants is significantly above any background reversion. Once

a yeast transformant has been identified, it is simple to return the responsible plasmid to *E. coli* (2, 6), where amplification and subsequent analysis of its physical structure are more tractable. In the experiments reported here, *TRP1*$^{+}$ *CDC28*$^{+}$ transformant yeast clones were obtained at a frequency of 0.04% of the *TRP1*$^{+}$ transformants. Plasmids from these clones were reisolated in *E. coli* and shown to be independently capable of complementing a *cdc28* mutation.

The first part of the cloning entails the isolation of sequences capable of complementing a mutation in a specific gene. An equally important step is to check the identity of the sequences because complementation does not alone prove that the cloned sequences actually correspond to the gene being sought. Suppression of mutations by secondary mutations at remote loci has been reported (19, 20). Although secondary mutations, per se, are unlikely in this case, other phenomena pertaining to secondary loci, such as dosage or sequence context effects, are of potential concern because the copy number and transcriptional efficiency of the plasmids are not precisely known. Therefore, some direct proof of identity of the cloned sequences is demanded. YRp7 has a feature that has proved useful to this end: i.e., its chronic instability as an autonomous plasmid in yeast (6). Stabilization of the plasmid-associated phenotypes by integration into chromosomal DNA allows such events to be selected simply on the basis of colony size under restrictive conditions. The position of the integrated plasmid in the genome can then be mapped by conventional genetic analysis (16) and shown to be linked to the locus being sought. In the work reported here, we demonstrate a novel linkage between *trp1*, a marker associated with the vector, and *tyr1*, a gene closely linked to *cdc28*. Because site-specific integration presumably can only occur if DNA sequence homology exists, this establishes the identity between the plasmid-associated sequences and the chromosomal sequences at the site of integration. One potential flaw in this argument is that a normally cryptic gene with sequence homology to *cdc28* might complement the primary mutation for reasons related to its being contained in a recombinant plasmid. In such a case, a recombination event resulting in integration at the primary locus might be possible, invalidating the above interpretation. For *cdc28*, we can rule out an error of this type because the insert sequences are unique in the yeast genome (inferred from Southern blots; data not shown). In addition to the above considerations, the physical linkage of sequences capable of complementing both *cdc28* and *tyr1* mutations in plasmid YRp7·*CDC28*(1) confirms the identity of the cloned sequences because the genetic linkage of these loci has already been established (refs. 4 and 7 and unpublished data). The "integration" technique described above should prove a useful addition to the means presently available for identifying cloned sequences (21–24) because it requires only that a mapped mutation be available. The presence of repetitive DNA elements on a plasmid may interfere with the mapping.

We believe that the broad applicability of yeast transformation cloning to genes whose products are uncharacterized makes it promising as a general means of identifying the products of such genes. With cloned genes, by using the procedures available, it is possible to isolate homologous mRNA sequences. Such RNAs should be translatable *in vitro*, directing the synthesis of the correspondent polypeptides, which can then be characterized. *cdc28* is a gene of particular interest because its function is essential for the step of the yeast cell cycle at which division is controlled (25). Identifying and characterizing the products of the genes that must function at this crucial point would be a major advance in understanding how division is controlled in eukaryotic cells.

Finally, the cloning of both *cdc28* and *tyr1* on a fragment of DNA 12.25 kb long establishes some variables relevant to genome structure. Because these two loci are separated by 7 meiotic map units, this result fixes a maximum equivalance of 1.75 kb per map unit for this region. Because the other cloned *cdc28* sequences are a subset of YRp7·*CDC28*(1), which contains both *tyr1* and *cdc28*, one can establish the relative orientation of the two genes within the cloned fragment of each with respect to the centromere of chromosome II.

We thank R. W. Davis for the use of YRp7. These studies were supported by National Institutes of Health grants to Benjamin D. Hall. S.I.R. acknowledges the support of American Cancer Society Postdoctoral Fellowship PF-1218; K.A.N. acknowledges support of a Jane Coffin Childs Postdoctoral Fellowship.

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