MOLECULAR AND CELLULAR BIOLOGY, Sept. 1989, p. 4096–4098
0270-7306/89/094096-03$02.00/0

Vol. 9, No. 9

Integration of an Aberrant Retrotransposon in *Saccharomyces cerevisiae*

CHARLES M. WILKE AND SUSAN W. LIEBMAN*

Laboratory for Cell, Molecular, and Developmental Biology, Department of Biological Sciences, University of Illinois at Chicago, Box 4348, Chicago, Illinois 60680

Received 24 March 1989/Accepted 12 June 1989

We describe an atypical composite Ty1 element that apparently resulted from the concurrent integration of two complete elements. A portion of the central region of one of these elements was inverted between two long terminal repeats. Inversions of this type have been detected among unintegrated retroviral circles. It now appears that such intermediates can be incorporated into the genome.

Unintegrated retroviral or retrotransposon DNA exists in several forms which differ in topology, configuration, and content (27). Initially, reverse transcription within the cytoplasmic virus particle results in a linear DNA duplex with a long terminal repeat (LTR) at each end. Some of this linear DNA is transported to the nucleus, where it appears to act as a substrate in proviral integration reactions (2, 5, 7). A portion of the linear DNA is also converted to circular forms, the more abundant of which consist of monomers containing one or two LTRs. Other, less abundant forms of retroviral or retrotransposon DNA, including dimeric circles (11) and monomeric circles with deletions and inversions (21), have also been characterized. Various mechanisms, including autointegration of transposition intermediates, can be invoked to explain the origin of the abnormal intermediates. These aberrant forms are considered dead-end byproducts of the retrotransposition process (17). In this paper, we provide the first evidence that these abnormal retrotransposon intermediates can be incorporated into the genome.

While characterizing 55 spontaneous canavanine-resistant mutations caused by transposition of the *Saccharomyces cerevisiae* retrotransposon Ty (C. Wilke, S. Heidler, N. Brown, and S. Liebman, Genetics, in press), we identified an atypical Ty1 element (Ty1-84) that apparently resulted from the concurrent integration of two complete elements into the *CAN1* gene (10). Tandem Ty elements in yeast strains (3) have been described and are thought to result from intergenic recombination between adjacent terminal LTRs (deltas) of two Tys inserted near each other in direct orientation (12). The composite Ty1-84 element found here differed from previously described tandem Ty elements by an inversion of a portion of its central region (epsilon) between two oppositely oriented deltas (Fig. 1). Inversions of this type have been detected among unintegrated retroviral and *copia* circles (6, 22, 26).

Ty1-84 was originally identified by Southern analysis (24) as a 12-kilobase (kb) insertion in the *CAN1* region of the canavanine-resistant strain YE84. The entire 12-kb Ty1-84 element was cloned as a 17.5-kb *Bam*HI fragment (pTy1-84) by integration and excision (25) with a pUC9-based vector containing the *TRP1* and *CAN1* yeast genes (C. Wilke et al., submitted). Figure 1B and C show the general structure of the composite Ty1-84 at its integration site in the *CAN1* locus. DNA sequences from restriction fragments of Ty1-84 cloned into mp18 or pUC9 were determined by the dideoxy-chain termination method (18). Figure 1A shows a typical Ty1 element with several restriction sites that are generally conserved. The 5-base-pair (bp) repeats at either end of Ty1-84 (Fig. 1B) (to the left of delta 1 and the right of delta 4) were derived from positions 58 to 62 at the 5′ end of the *CAN1* open reading frame (10) and are indicative of a normal Ty transposition event (8). Sequence analysis of the internal Ty1-84 delta elements and much of the surrounding DNA revealed two complete delta elements separated by about 820 bp of Ty epsilon DNA normally found in this region (4). The two internal deltas are identical and can be differentiated only by their neighboring epsilon sequences. These deltas do not contain *Xho*I sites, whereas the deltas at the ends of Ty1-84 do.

The 5 bp of DNA at the delta-epsilon junctions are completely conserved among normal Ty elements (4) (Fig. 1A and D). This typical 5-bp sequence is also found to the left of the Ty1-84 delta 2, whereas the 5 bp to the right of delta 2 are derived from the epsilon region and correspond to positions 1147 to 1151 of Ty912 (4) (Fig. 1B and C). These 5 bp and the neighboring DNA are inverted compared with delta 2. The same 5 bp are repeated at the right end of delta 3 in their normal orientation and position with respect to the rest of the epsilon sequences. The repetition of 5 bp surrounding these delta elements is indicative of a transposition event. The 5 bp to the left of delta 3 are those normally present at the delta-epsilon junctions from the 5′ end of Ty elements but, like delta 3, they are inverted relative to delta 2.

One possible origin of Ty1-84 could be a normal transposition event followed by a gene conversion of the transposed Ty with a preexisting genomic template having a structure similar to that of Ty1-84. Such a template could have arisen from multiple transposition and recombination events. To test this possibility, Southern analysis (24) was used to determine if Ty elements with structures similar to that of Ty1-84 already existed in the parental canavanine-sensitive strain, SL854-12C. Figure 2A compares the *Bgl*II-*Pvu*II Ty hybridization spectra of YE84 and SL854-12C. The *Bgl*II and *Pvu*II sites in the region probed are highly conserved among Ty elements, as is evident from the hybridization pattern. The 1.2-kb *Bgl*II-*Pvu*II fragment is characteristic of unrearranged Ty elements, and its intensity reflects the approximately 30 copies of Ty1. Ty1-84 contains both the normal 1.2-kb fragment and a 1.0-kb fragment indicative of

* Corresponding author.

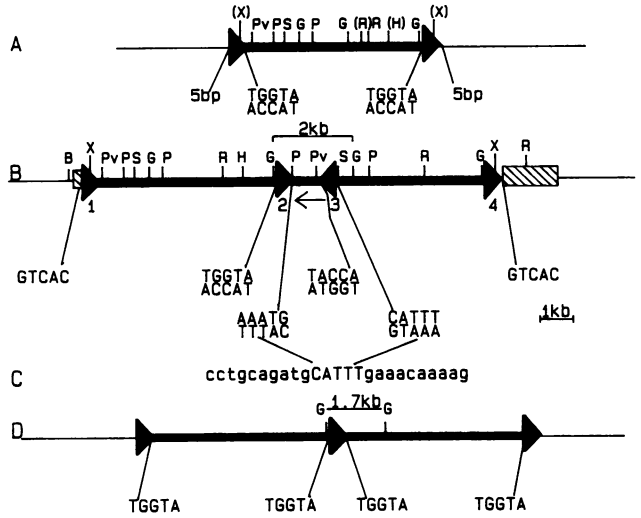

FIG. 1. Structure of Ty1-84 in the *CAN1* region of YE84. The closed triangles represent the delta elements (LTRs) and indicate the direction of transcription. Restriction enzymes: X, *Xho*I; Pv, *Pvu*II; P, *Pst*I; G, *Bgl*II; B, *Bam*HI; R, *Eco*RI; H, *Hin*dIII; S, *Sph*I. (A) Typical Ty1 element (adapted from reference 28). Each element contains two long terminal direct repeats of about 335 bp (deltas) flanking a central epsilon region of 5.3 kb. The 5-bp repeats at each end of the element derived from the integration site are shown. Parentheses around restriction enzyme symbols indicate that these sites are variable. The 5-bp sequences at the delta-epsilon junctions are taken from reference 4. (B) Ty1-84 at the *CAN1* locus. The cross-hatched boxes represent the *CAN1* locus. The numbers designate the deltas as described in the text. The inverted region is indicated by the arrow. The sequences represent the *CAN1*-delta or delta-epsilon junctions. The 2-kb *Bgl*II restriction fragment discussed in the text is indicated. (C) Epsilon sequences from reference 4. The 5 bp repeated in Ty1-84 are indicated by uppercase letters. (D) Tandem Ty with predicted delta-epsilon junction sequences. The 1.7-kb *Bgl*II fragment discussed in the text is indicated.

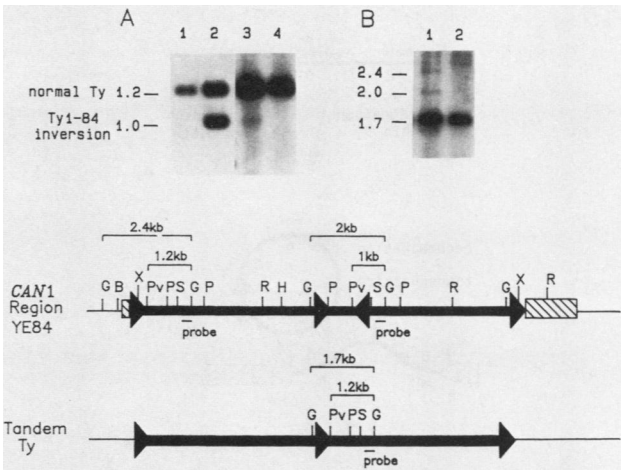

FIG. 2. Southern blot analysis of SL854-12C and YE84 (A and B) and position of the 354-bp *Sau*3A fragment used as a probe for both blots (bottom). The delta elements and restriction enzymes are indicated as described in the legend to Fig. 1. (A) *Bgl*II-*Pvu*II blot analysis of SL854-12C and YE84 (see text). Yeast genomic DNA was isolated as previously described (20), and plasmid DNA was isolated and purified by the alkaline lysis and CsCl methods (14). The DNA was digested, run, transferred to Hybond-N (Amersham Corp.) or GeneScreen Plus (Du Pont Co.), and hybridized according to the specifications of the manufacturers. All DNAs were digested with *Bgl*II and *Pvu*II. Lane 1, Plasmid pNN116, which contains Ty1-D15, a normal Ty element (19); lane 2, plasmid pTy1-84, which contains a rearranged and normal Ty element; lane 3, genomic DNA from YE84; lane 4, genomic DNA from SL854-12C, the precursor of YE84. (B) *Bgl*II blot analysis of YE84 and SL854-12C. Lane 1, YE84 genomic DNA; lane 2, SL854-12C genomic DNA. In lane 1, the new 2- and 2.4-kb bands attributable to Ty1-84 can be seen, whereas they are absent from SL854-12C (see restriction map and text). The sizes (in kilobases) of the bands homologous to the probe are shown to the left of each autoradiograph.

the internal inversion. It can be seen that whereas the YE84 genome carries the Ty rearrangement known to exist in the *CAN1* region (Ty1-84), no such Ty structure is detected in SL854-12C. The *Bgl*II digestions shown in Fig. 2B gave similar results. It is predicted that typical tandem Ty elements will release an internal 1.7-kb *Bgl*II fragment homologous to the same probe, whereas a tandem Ty similar to Ty1-84 will have a 2-kb *Bgl*II fragment. A 2-kb band is seen for YE84 but not for SL854-12C. The new 2.4-kb fragment seen in YE84 was predicted from the restriction map of the *CAN1*::Ty1-84 region.

The data presented above demonstrate that the origin of Ty1-84 did not involve conversion of a Ty insertion at *CAN1* by a preexisting Ty element, because the required template was not present in the genome prior to the transposition. The restriction and sequence data reveal that Ty1-84 contains a structure similar to that of a class of aberrant monomeric circular forms characterized from a number of retroviruses and retrotransposons (6, 21, 22, 26). These aberrant circles contain either two oppositely oriented LTRs separated by an inversion of the retroviral genome or a single LTR juxtaposed to a deletion of the genome. Both of these structures can arise when a transposition intermediate integrates into itself. Shoemaker et al. (22) found that up to 20% of the circular forms of Moloney murine leukemia virus contain internal deletions or inversions, suggesting that autointegration of transposition intermediates is common. Eichinger and Boeke (5) recently reported that a large proportion of Ty circles consist of deleted forms presumably originating from autointegration. Although none were found by Eichinger and Boeke (5), it seems likely that aberrant circular forms of Ty containing inversions also arise from the same process and that such a molecule was the precursor of the Ty1-84 element. Figure 3 shows a simple potential pathway for the origin of Ty1-84 in which a transposition event is coupled to homologous recombination with an aberrant circular intermediate.

High levels of genetic recombination between retroviruses are well documented (13) and often important in the progression of the diseases these viruses cause (15). The process of reverse transcription appears to be responsible for a good deal of retroviral recombination (1, 16, 23, 29). However, there is also evidence that some genetic exchange can result from the interaction of unintegrated retroviral DNA subsequent to reverse transcription, since dimeric circles and molecules apparently derived from this DNA have been found (11, 22). Recent evidence suggests that these types of molecules may occasionally recombine into the genome when components of the retroviral integration reaction are mutated (9, 13a). Our analyses of the Ty1-84 element suggest that complex Ty rearrangements may be generated in *S. cerevisiae* by homologous recombination of aberrant intermediates into recently transposed Ty elements. The involvement of aberrant intermediates in genetic exchange among retroviruses may contribute to the reorganization and evolution of retroviral genomes.

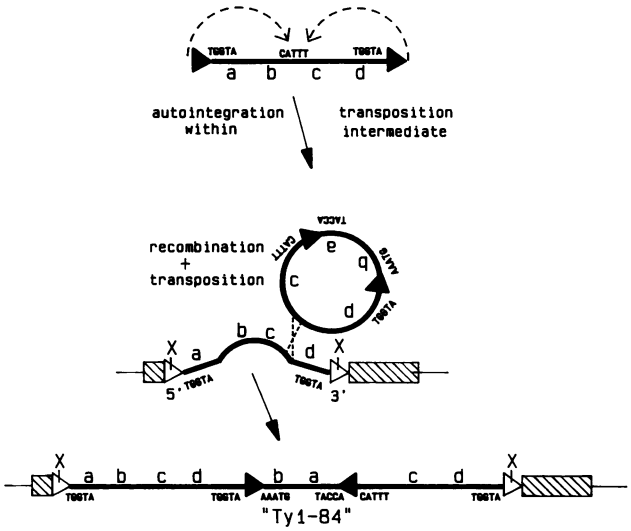

FIG. 3. Hypothetical pathway leading to the formation of Ty1-84. Triangles represent deltas; a, b, c, and d orient the epsilon sequences; and the cross-hatched boxes represent the *CAN1* locus. The small 5-bp sequences represent the epsilon-delta junction repeats and the target of autointegration found in Ty1-84. According to the model, transposition accompanies homologous recombination with an aberrant monomeric circular Ty element. First, autointegration produces an aberrant monomeric transposition intermediate. This monomer then recombines with a Ty element either before or after it transposes into the *CAN1* locus. The open and closed deltas imply sequence differences. The pattern of *Xho*I sites (X) in the Ty1-84 deltas is easily rationalized by these series of events.

We are especially grateful for the initial isolation and characterization of strain YE84 by Steve Heidler and the construction of SL854-12C by Paul Sutton. We thank Jef Boeke and Karen Downs for their helpful comments on the manuscript and Stephen Goff for helpful discussion. We also thank Leslie Lobel, John Murphy, and Stephen Goff for sharing unpublished data.

This work was supported by Public Health Service grant GM36900 from the National Institutes of Health.

LITERATURE CITED

1. **Boeke, J. D., C. A. Styles, and G. R. Fink.** 1986. *Saccharomyces cerevisiae SPT3* gene is required for transposition and transpositional recombination of chromosomal Ty elements. Mol. Cell. Biol. **6**:3575–3581.
2. **Brown, P. O., B. Bowerman, H. E. Varmus, and J. M. Bishop.** 1987. Correct integration of retroviral DNA *in vitro*. Cell **49**:347–356.
3. **Cameron, J. R., E. Loh, and R. W. Davis.** 1979. Evidence for transposition of dispersed repetitive DNA families in yeast. Cell **16**:739–751.
4. **Clare, J., and P. Farabaugh.** 1985. Nucleotide sequence of a yeast Ty element: evidence for an unusual mechanism of gene expression. Proc. Natl. Acad. Sci. USA **82**:2829–2833.
5. **Eichinger, D., and J. D. Boeke.** 1988. The DNA intermediate in yeast Ty1 element transposition copurifies with virus-like particles: cell-free Ty1 transposition. Cell **54**:955–966.
6. **Flavell, A. J., and D. Ish-Horowicz.** 1983. The origin of extrachromosomal circular *copia* elements. Cell **34**:415–419.
7. **Fujiwara, T., and K. Mizuuchi.** 1988. Retroviral DNA integration: structure of an integration intermediate. Cell **54**:497–504.
8. **Gafner, J., and P. Philippsen.** 1980. The yeast transposon Ty1 generates duplications of target DNA on insertion. Nature (London) **286**:414–418.
9. **Hagino-Yamagishi, K., L. A. Donehower, and H. E. Varmus.** 1987. Retroviral DNA integrated during infection by an integration-deficient mutant of murine leukemia virus is oligomeric. J. Virol. **61**:1964–1971.
10. **Hoffmann, W.** 1985. Molecular characterization of the *CAN1* locus in *Saccharomyces cerevisiae*. J. Biol. Chem. **260**:11831–11837.
11. **Kung, H., P. R. Shank, J. M. Bishop, and H. E. Varmus.** 1980. Identification and characterization of dimeric and trimeric circular forms of avian sarcoma virus-specific DNA. Virology **103**:425–433.
12. **Liebman, S. W., and S. Picologlou.** 1988. Recombination associated with yeast retrotransposons, p. 63–90. *In* Y. Koltin and M. J. Leibowitz (ed.), Viruses of fungi and simple eukaryotes. Marcel Dekker, Inc., New York.
13. **Linial, M., and D. Blair.** 1984. Genetics of retroviruses, p. 649–784. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

13a. **Lobel, L. I., J. E. Murphy, and S. P. Goff.** 1989. The palindromic LTR-LTR junction of Moloney murine leukemia virus is not an efficient substrate for proviral integration. J. Virol. **63**: 2629–2637.

14. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Overbaugh, J., N. Riedel, E. A. Hoover, and J. I. Mullins.** 1988. Transduction of endogenous envelope genes by feline leukaemia virus in vitro. Nature (London) **332**:731–734.
16. **Panganiban, A. T., and D. Fiore.** 1988. Ordered interstrand and intrastrand DNA transfer during reverse transcription. Science **241**:1064–1069.
17. **Panganiban, A. T., and H. M. Temin.** 1984. Circles with two tandem LTRs are precursors to integrated retrovirus DNA. Cell **36**:673–679.
18. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
19. **Scherer, S., and R. W. Davis.** 1980. Recombination of dispersed repeated DNA sequences in yeast. Science **209**:1380–1384.
20. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Laboratory course. Manual for methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Shoemaker, C., S. Goff, E. Gilboa, M. Paskind, S. W. Mitra, and D. Baltimore.** 1980. Structure of a cloned circular Moloney murine leukemia virus DNA molecule containing an inverted segment: implications for retrovirus integration. Proc. Natl. Acad. Sci. USA **77**:3932–3936.
22. **Shoemaker, C., J. Hoffmann, S. P. Goff, and D. Baltimore.** 1981. Intramolecular integration within Moloney murine leukemia virus DNA. J. Virol. **40**:164–172.
23. **Skalka, A. M., L. Boone, R. Junghans, and D. Luk.** 1982. Genetic recombination in avian retroviruses. J. Cell. Biochem. **19**:293–304.
24. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
25. **Stiles, J. I.** 1983. Use of integrative transformation of yeast in the cloning of mutant genes and large segments of contiguous chromosomal sequences. Methods Enzymol. **101**:290–300.
26. **Van Beveren, C., E. Rands, S. K. Chattopadhyay, D. R. Lowy, and I. M. Verma.** 1982. Long terminal repeat of murine retroviral DNAs: sequence analysis, host-proviral junctions, and preintegration site. J. Virol. **41**:542–556.
27. **Varmus, H. E.** 1983. Retroviruses, p. 411–503. *In* J. A. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
28. **Williamson, V. M.** 1983. Transposable elements in yeast. Int. Rev. Cytol. **83**:1–25.
29. **Xu, H., and J. D. Boeke.** 1987. High-frequency deletion between homologous sequences during retrotransposition of Ty elements in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **84**:8553–8557.