A model for replication of the ends of linear chromosomes

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ABSTRACT

Linear chromosomes possessing internal repeats of their terminal sequences can form intramolecular crossed-strand exchanges that allow replication of the chromosome ends. Evidence is discussed that such a mechanism may be utilized during replication of herpes simplex virus DNA and during replication of macronuclear DNA from the hypotrichous ciliate *Oxytricha*.

RESULTS AND DISCUSSION

The known DNA polymerases are capable of adding nucleotides to the 3' OH terminus of a pre-existing polynucleotide chain. Unlike RNA polymerases, they apparently are not capable of initiating such chains *de novo*. RNA-synthesizing enzymes are now thought to initiate DNA replication by providing RNA "primers" which DNA polymerase can extend.^{1,2} A newly replicated linear DNA will therefore possess an RNA primer at the 5' end of its daughter strand. As noted by Watson, excision of this terminal primer leaves a gap which DNA polymerase can not fill.³ Unfilled gaps can not be tolerated since shorter DNA molecules would arise at each generation. Linear chromosomes should therefore possess some special nucleotide sequence allowing replication of their ends. Linear bacteriophage chromosomes frequently have complementary sequences at both ends, for example.¹ This provides a mechanism for replication of the chromosome ends by allowing cyclization (e.g. phage λ) or concatamerization (e.g. T7) during replication.³ Palindromes (two-fold symmetric sequences) at the ends of a linear chromosome have been suggested as an alternate solution.⁴ Here, I demonstrate that internal repeats of the terminal sequences can also allow replication of the ends of a linear DNA.

A linear DNA with an internally located repeat of the terminal sequences is shown in figure 1a. The sequences at the two termini are assumed to be identical, although this is not required. The internal sequence thus comprises an inverted repeat of the right terminal sequence and a non-inverted repeat of the left. Following nicking by a site-specific endonuclease

Figure 1. Model for replication of the ends of linear chromosomes. a, Linear chromosome with identical terminal sequences and a single internal repeat of this sequence. Heavy lines indicate potential sites for RNA primers or gaps resulting from their excision. Actual molecules would each possess a primer only at the 5' end of their daughter strand. Primers have been shown shorter than the terminal sequence although this is not required. Arrow indicates the position to be nicked by site-specific endonuclease; b, c, Crossed-strand exchanged structures formed from (a) after nicking. In (b) the right 5' end of the linear chromosome and in (c) the left 5' end has been folded into the interior of the molecule. Gaps resulting from primer excision may now be repaired by DNA polymerase and ligase; d, e, Structures resulting from repair of (b) and (c) respectively. Nicking either (d) or (e) at "1" yields a fully-replicated, nicked, linear chromosome with the original sequence. Nicking (d) at "2" yields a nicked linear DNA in which the region of the chromosome to the right of the internal repeat has been inverted. Nicking (e) at "2" yields a nicked circle containing the left portion of the chromosome and a linear containing the right.

(Fig. 1a) the molecule can undergo either of two intramolecular crossed-strand exchanges (Figs. 1b & c). Either 5' end of the linear DNA can thus come to lie at an internal site adjacent to the 3' OH terminus created by nicking. Exonucleolytic removal of RNA primers and gap filling by DNA polymerase can proceed freely in such a configuration. Ligation results in a closed structure (Figs. 1d & e). Nicking by the endonuclease invoked above can then allow reversal of the crossed-strand exchange process, yielding a nicked linear which ligase may seal to produce a completely replicated molecule. Internal repeats (inverted or not) of a terminal sequence can thus allow replication of that terminus.

Additional possibilities arise when internal repeats of both ends are present and adjacent to each other (Fig. 2a). Such a molecule can undergo two simultaneous crossed-strand exchanges without prior nicking (Fig. 2b). Repair of gaps left by primer excision can then proceed as in the previous case. Two nicks are now required to re-form a linear molecule (Fig. 2c).

The crossed-strand exchanged structures suggested here can all arise with no net loss of base-pairing, so long as simple geometric constraints are met.⁵ For example, the structures shown in figures 2b and 2c require that the internal repeats together contain an integral number of helical turns. Nonetheless, the required structures will rarely arise spontaneously due to the configurational entropy lost during their formation.⁶ This difficulty can be removed by postulating a protein which binds tightly to the terminal sequences and their internal repeats, holding all of these in close proximity with the required orientation. Formation of the structures discussed in figure 2 would presumably be catalyzed by a two-fold symmetric protein composed of four identical subunits, each subunit binding to one terminal sequence or internal repeat. The required site-specific endonuclease might also reside in this same protein. Such a protein could also catalyze formation of crossed-strand exchanges of the type discussed in connection with figure 1, given identical terminal sequences and adjacent internal repeats (Fig. 2a). Symmetry dictates that a tetramer of this type could not function as a strand-specific endonuclease. As discussed in the legends to figures 1 and 2, this should result in chromosomal aberrations, most notably inversions of the regions on either side of the internal repeats.

Although the model presented is speculative, some data consistent with it are known. Several workers have reported that the linear chromosome of herpes simplex virus possesses adjacent internal repeats of its terminal sequences.^{7,8,9} The structure apparently differs from that shown in figure

Figure 2. Model for replication of the ends of linear chromosomes.
 a, Linear chromosome with identical terminal sequences and adjacent internal repeats of this sequence. The internal repeats comprise a palindrome (two-fold symmetric sequence). Other symbols as in figure 1;
 b, Structure arising from (a) by two crossed-strand exchanges. The 5' primer sites are now adjacent to free 3' OH termini and may be excised and repaired. Note that either terminal sequence can be paired with a given internal repeat;
 c, Closed structure resulting from primer excision and repair. Introduction of nicks at "2" and "3" yields a fully replicated linear chromosome with the original sequence. Nicking at "1" and "4" yields a linear chromosome with both the right and left hand regions inverted. Any other combination of two nicks at the sites indicated results in a doubly-nicked circular molecule.

2a only in that the terminal sequences are partially homologous rather than identical. Further the sequences on either side of the internal repeats are found in all four possible orientations, suggesting frequent inversions of the type discussed above.¹⁰

Macronuclear DNA from the ciliate *Oxytricha* also has a structure consistent with the proposed replication scheme. *Oxytricha* macronuclear DNA consists of linear DNA pieces with a number average molecular weight of 2.1×10^6 daltons.^{11,12} Roughly 17,000 different types of pieces are found in the mature macronucleus, each present about 1000 times per nucleus.¹³ Wesley has reported that most and possibly all of the macronuclear DNA pieces possess adjacent, internally located, inverted repeats of their terminal sequences.¹² Preliminary data suggest that the two termini of a DNA piece are identical to each other (G. Herrick, personal communication), again suggesting a sequence of the type shown in figure 2a. Additionally, Wesley finds nicks or single-stranded gaps at specific sites within the *Oxytricha* internal repeats, implying the presence of a site-specific endonuclease, as required by the model.

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