

NOTES

Nucleotide Sequence of an Unequal Sister Chromatid Exchange Site in a Mouse Myeloma Cell Line

DANIEL R. KATZENBERG, SHERMAINE A. TILLEY,† AND BARBARA K. BIRSHEIN*

Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461

Received 15 July 1988/Accepted 7 December 1988

The mouse myeloma cell line MPC 11 carries two C γ 2a immunoglobulin heavy-chain genes on the expressed chromosome, a duplication shown to have occurred through unequal sister chromatid exchange (USCE). In the present report, we present the nucleotide sequence of the USCE joint and show that both breaks occurred within tracts of repeated TC dinucleotides. Additional TC dinucleotide tracts and two oligonucleotide segments (N sequences) were inserted at the USCE site.

A single copy of each of the eight immunoglobulin heavy-chain constant region genes is present in the germ line of BALB/c mice. The myeloma cell line MPC 11 carries a tandem duplication of the C γ 2a gene on the expressed chromosome (Fig. 1). The expressed chromosome contains a deletion of γ 2a-associated tandem repeats, while its homolog maintains a germ line configuration in this region; since both copies of γ 2a involved in the duplication carry the deletion, unequal sister chromatid exchange (USCE), rather than mitotic recombination between homologs, is the molecular mechanism responsible for the duplication (13). Furthermore, USCE appears to be an ongoing process in MPC 11; variants in heavy-chain production derived from MPC 11 carry the reciprocal products of further USCE (either loss or further amplification of the duplicated γ 2a gene).

The DNA sequences of the germ line regions involved in the crossover, one located approximately 3 kilobases 3' to C γ 2b and the other the same distance 3' of C γ 2a, are virtually identical (15), strongly suggesting homologous pairing of the two regions as an initial step. The size of the USCE joint, however, implies that if crossing over followed precise pairing, the nicks in the two strands did not occur at equivalent positions. We measured an offset of about 50 nucleotides between the two cuts. The crossover site was further localized by genomic Southern blot analysis within a region of juxtaposed tracts of TC and TG dinucleotide repeats. Alternating purine-pyrimidine tracts, such as (TG)_n, may assume a Z-DNA conformation (11), whereas regions of TC simple sequence may assume a novel non-B, non-Z, triplex structure (10) and have been suggested to be structural elements of S1 nuclease-hypersensitive sites (3).

To precisely localize the site of recombination, we sought to clone and sequence the USCE joint from a cell line carrying the C γ 2a duplication. The USCE joint was cloned from the MPC 11 variant ICR 16, the γ 2a genes (and hence the USCE joint) of which are amplified approximately 5- to 10-fold (13). Since previous cloning attempts with large fragments in cosmid and phage vectors were unsuccessful,

perhaps due to recombinogenic properties of the USCE joint, we sought to clone this region as a small fragment in a plasmid vector. In order to enrich the library for the USCE joint, a double-fractionation strategy was employed. The USCE joint was present on a 6.4-kilobase *Bgl*III fragment and on an internal *Hind*III fragment of 2.5 kilobases (Fig. 1a). Genomic DNA was digested with *Bgl*III and size fractionated; DNA of the appropriate size was recovered, digested with *Hind*III, and size fractionated a second time. DNA of the desired size range was recovered and ligated with *Hind*III-digested pBR322. Transformants were screened with γ 2IVS 3.6 (Fig. 1). Two independent positive colonies, NJ25 and NJ41, were isolated; restriction maps of the plasmid they carried were consistent with the map of the USCE joint determined by genomic Southern blot analysis.

The sequence of the USCE joint, present on the 700-base-pair (bp) *Hind*III-*Eco*RI fragment (Fig. 1a), was determined by the chemical modification method (9). Both strands were read from the *Hind*III site of γ 2a-3' to the *Nsi*I site present in γ 2b-3' sequences; the sequences of NJ25 and NJ41 were identical (Fig. 1b). As expected, the sequence was identical to γ 2a-3' for a maximum of 49 bases at its 5' end and identical to γ 2b-3' for a maximum of 106 bp 5' to the *Nsi*I site. Between the two germ line sequences lay at least 64 bp with the general structure (TC)₁₁ N₆ (TC)₅ N₈ (TC)₉. The N segments were not present within either γ 2a-3' or γ 2b-3'. In γ 2a-3', the USCE site lay either at the 3' edge of or somewhere within the TC tract. Similarly, the break in γ 2b-3' was either at the 5' edge of or anywhere within the TC dinucleotide repeat.

The potentially Z-DNA-forming TG-rich region of γ 2a-3' was eliminated from the USCE joint, whereas that of γ 2b-3' lay at least 16 bases distal to the USCE site. Studies on the *Ustilago* RecI protein (5-7) have shown that Z-DNA segments are important in the pairing step of the recombination catalyzed by this enzyme, while other studies (14) identified a role for TG sequences in yeast meiotic recombination. In the MPC 11 USCE, the potential Z-DNA may serve as a recognition site for recombinases involved in the pairing of the sister chromatids with the actual nicks introduced some distance away.

The mechanism of the USCE appears to be complex. In both clones which were sequenced (NJ25 and NJ41), there

* Corresponding author.

† Present address: Department of Developmental and Structural Biology, The Public Health Research Institute, New York, NY 10016.

7. **Kmiec, E. B., and W. K. Holloman.** 1986. Homologous pairing of DNA molecules by *Ustilago* RecI protein is promoted by sequences of Z-DNA. *Cell* **44**:545-554.
8. **Landau, N. R., D. G. Schatz, M. Rosa, and D. Baltimore.** 1987. Increased frequency of N-region insertion in a murine pre-B-cell line infected with a terminal deoxynucleotidyl transferase retroviral expression vector. *Mol. Cell. Biol.* **7**:3237-3243.
9. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* **65**:499-560.
10. **Mirkin, S. M., V. I. Lyamichev, K. N. Drushlyak, V. N. Dobrynin, S. A. Filippov, and M. D. Frank-Kamenetskii.** 1987. DNA H form requires a homopurine-homopyrimidine mirror repeat. *Nature (London)* **330**:495-497.
11. **Nordheim, A., E. M. Lafer, L. J. Peck, J. C. Wang, B. D. Stollar, and A. Rich.** 1982. Negatively supercoiled plasmids contain left-handed Z-DNA segments as detected by specific antibody binding. *Cell* **31**:309-318.
12. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463-5467.
13. **Tilley, S. A., and B. K. Birshtein.** 1985. Unequal sister chromatid exchange: a mechanism affecting Ig gene arrangement and expression. *J. Exp. Med.* **162**:675-694.
14. **Treco, D., and N. Arnheim.** 1986. The evolutionarily conserved repetitive sequence $d(TG \cdot AC)_n$ promotes reciprocal exchange and generates unusual recombinant tetrads during yeast meiosis. *Mol. Cell. Biol.* **6**:3934-3947.
15. **Weinreb, A., D. R. Katzenberg, G. L. Gilmore, and B. K. Birshtein.** 1988. A site of unequal sister chromatid exchange contains a potential Z-DNA-forming tract. *Proc. Natl. Acad. Sci. USA* **85**:529-533.