Sequence at insertion site of E.Tn retrotransposon into an immunoglobulin switch region suggests a role for switch recombinase

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E.Tn (for early transposon-like) elements are a family of murine retrotransposon-like elements (1). These 5.6 kb sequences are flanked by two 330 bp long terminal repeats (LTRs) and are present in approximately 1000 copies in the mouse genome (2). These elements are transcribed in early embryogenesis and in plasmacytomas, but they contain no significant open reading frames and are therefore unable to code for proteins involved in their transposition. Nevertheless, E.Tn elements have been shown to have integrated into different loci in four cases: three in the plasmacytoma P3 and its derivatives (3–5), and once into the *T* locus (6).

Two of the insertions of E.Tn in plasmacytoma P3 DNA have occurred in immunoglobulin switch (S) regions. The insertion into the $\gamma 1$ switch region appears to have occurred via a normal transposition process: the LTRs are identical and the insertion resulted in the duplication of six base pairs of the target sequence (4). Characterization of the 5' end of the insertion into an S $\gamma 2a$ region revealed a deletion of the first 631 bp of the E.Tn element, including the 5' LTR. In order to further understand this deletion and to identify the S $\gamma 2a$ target sequence, we cloned the 3' end of the insertion.

We used polymerase chain reaction (PCR) to amplify the sequences at the 3' end of the insertion. The 5' primer was complementary to part of the LTRs of the E.Tn element found in the $\gamma 1$ insertion. The 3' primer corresponded to downstream S $\gamma 2a$ sequences. Although E.Tn elements are present in many copies in the genome, only the sequence representing the insertion into the S $\gamma 2a$ region would be efficiently amplified. The amplified product was then cloned into M13 using *Xho*I and *Bgl*II sites (both orientations) and sequenced with Sequenase and ddNTPs.

The amplified sequence contained the last 58 bp of the E.Tn LTR followed by 314 bp of S $\gamma 2a$ sequences to the *Bgl*II site (Fig. 1). Comparing this sequence to the 5' end of the insertion revealed a duplication of 70 bp of the S $\gamma 2a$ target sequences. Duplication of sequences of this length is not characteristic of the transposition mechanism, but has been seen as the result of immunoglobulin heavy chain switch recombination (7). In the heavy chain switch recombination event, the target DNA may be nicked in two places, generating staggered ends, and subsequent DNA synthesis results in the duplication of these sequences on the order of hundreds of base-pairs. The cutting and recombination events tend to occur near conserved pentameric sequences in switch

regions which may be acting as signal sequences for the recombination event. The sequences which delimit the deletion of 5' end of the E.Tn element (AAGCT—underlined in Fig. 1) are homologous to one of these signal pentamers. Based on these observations, we propose that the switch recombination machinery played a role in the insertion of the E.Tn element into the S $\gamma 2a$ region, not only by cutting and duplicating the S $\gamma 2a$ region but also by cutting the E.Tn double-stranded DNA substrate.

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Figure 1. Sequences at the insertion site of an E.Tn element into S $\gamma 2a$. E.Tn sequences are shown in italics and numbered as in reference 2. S $\gamma 2a$ sequences are numbered arbitrarily. The sequences 5' to the 4900 bp break are from reference 4; those reported here are 3' to the 4900 bp break (GenBank accession number: M55665).

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