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ORIENTATION EFFECT IN IS30 TRANSPOSITION – ROLE OF SUBTERMINAL SEQUENCES

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The *E. coli* element IS*30* belongs to a growing class of ISs known to transpose through an intermediate formed by abutting the inverted repeats (IR) of the element. Two observations suggest that IS*30* is "able to distinguish" between its ends resulting in a so called "orientation effect": (i) The activity of IS*30*-based compound transposons differs according to the relative (direct or inverse) orientation of the IS elements. (ii) IS*30* tends to integrate next to the end of other IS*30* copies and this IR-targeting event always results in a head-to-tail junction of the elements.

Here, we demonstrate that the enhancer elements previously identified in the subterminal regions of IS*30* have a crucial role in the orientation effect. These enhancers are located within the 51-bp internal part in left end (AAAC repeats) and the 17-bp internal part in the right end (GAGATAATTG box) and all are required for efficient joining the ends. While both IRs deprived of the enhancer sequences are able to form head-to-head junctions, identical ends longer than ca. 65-bp, harbouring all enhancer elements, always form head-to-tail junctions in vivo. The fact that head-to-head junctions of longer ends are detectable in "in vitro" assays suggests that the lack of any types of enhancer elements significantly reduces, but does not completely inhibit joining the identical IS*30* ends and points to a second factor playing a key role in the full repression of formation of head-to-head junctions in vivo. We show that long palindromes cause serious replication defect of the replicon harbouring the joined identical ends longer than 65-bp. This effect together with the reduced efficiency of joining the identical ends due to the lack of either enhancer element can account for both the inactivity of the inverse compound transposons and the complete lack of head-to-head junctions observed in IR-targeting events.