oma virus strains[20], host range mutants of polyoma virus[21-23] and retroviruses[24].

Unlike the SV40 mutants with a deleted enhancer region, the *dpm*12 mutant retains all but four bases of the enhancer region intact. Therefore, the revertants described here allow identification of enhancer sequence elements that can best overcome the defects of an enhancer mutant. The new sequences created at the junctions of the different tandem duplications do not show any obvious homology, indicating that it is not these sequences *per se* which restore activity; in particular the patterns of alternating purines and pyrimidines destroyed by the *dpm*12 mutations are not recreated by the junction sequence.

The most striking feature of the *dpm*12 revertants is that a 15 bp region (the stippled areas in Fig. 1*b*) is shared by all the duplicatons, which suggests that this region plays a role in restoring activity to the *dpm*12 mutant. The hypothesis is strengthened by the fact that the sequences involved in the various duplications span 147 bp; these 147 nucleotides could have accommodated the duplications 71 bp and shorter (that is, 10 of the 18 revertants) without necessarily generating any common region. The sequence of this 15 bp region, TGTGGAAAGTCCCCA, contains the 'core' element (underlined) that was first identified on the basis of related sequences that are found among many enhancer elements[14]. (A second consensus sequence, the 'CACA' box[25], overlapping the 'core' element, is contained only partially in the 15 bp common region.) The consistent duplication of the 'core' region in 18 independent revertants suggests a critical function for this element in reactivating the *dpm*12 enhancer.

The structure of the *dpm*12 revertants are the result of two separate constraints: the reversion phenotypes selected for and the ease with which certain types of mutation can occur. In all likelihood no point revertants were isolated both because the *dpm*12 mutant carried four separate point mutations and because genomic rearrangements occur readily during SV40 replication[26-28]. To obtain revertants of the *dpm*12 mutant we selected directly for improved growth and infectivity of SV40, rather than for enhancer function. Such a selection probably places multiple constraints on the duplications that can best restore viability to the revertants. Therefore, the duplications described here are not necessarily those in which enhancer function has been maximized. Nevertheless, each revertant displays restored enhancer activity, indicating that the major defect of the *dpm*12 mutant is the lack of a healthy enhancer.

Duplications affect simultaneously two parameters: spacing between *cis*-acting elements and the sequences duplicated. Therefore, these parameters cannot be entirely independently analysed in these revertants. The three-fold difference in duplication size (45–135 bp) among the various *dpm*12 revertants suggests that if spacing requirements exist they are flexible. The fact that wild-type strains of SV40 also contain enhancer duplications of various sizes is consistent with this observation; in addition to the prototype 72 bp repeat of strain 776, other wild-type strains carry duplications ranging from 64 to 93 bp in length,[4,29-31]. Two features of the revertants, however, suggest that there is some duplication size preference: (1) those repeated sequences between 51 and 71 bp long seem to be more active as enhancers than the larger duplications (see Fig. 1*c*) and (2) the shortest duplication, *rd*45, is the least active of the 18 revertants.

The results described here indicate that the SV40 enhancer consists of multiple elements. The *dpm*12 mutant shows that two separate regions are important for enhancer function and the revertants show that loss of enhancer function in *dpm*12 can be restored by duplication of sequences not affected directly by the mutations (that is *rd*45–*rd*53, see Fig. 1). The consistent duplication of the 'core' region in each of the 18 revertants suggests that these sequences are compensating for and apparently acting independently of the mutated *dpm*12 sequences.

Our results show that a variety of duplications in a transcriptional control region can generate enhancers many times more active than the original. Perhaps strong viral enhancers evolved initially by duplication of weaker cellular enhancers.

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Erratum

Involvement of circular intermediates in the transfer of T-DNA from *Agrobacterium tumefaciens* to plant cells

Zdena Koukolíková-Nicola, Raymond D. Shillito, Barbara Hohn, Kan Wang, Marc Van Montagu & Patricia Zambryski

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This article was published with the sixth author's surname spelt incorrectly. It is shown correctly above.