The role of persistent nicks at the boundaries of interphase chromatin loops and their possible involvement in pathological gene rearrangements

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Still little is known about the signals marking the borders of interphase chromatin loops, beyond the vulnerability of the chromatin at these sites, and the possible involvement of specific, though heterogeneous sequences (S/MAR elements) in their anchorage to a putative nuclear matrix. The global disintegration of chromatin into ≥50 kb fragments as a result of protein denaturing treatments of nuclei prepared from healthy, non-apoptotic cells, considered indicative of loop arrangement, was discovered in the early '90s. The phenomenon seems particularly important because *(a)* it reflects regularities in the organization of higher order chromatin structure, *(b)* it appears to be interrelated with questions concerning genome instability, chemotherapy-associated gene rearrangements and apoptosis.

Applying biophysical and molecular biological techniques (e.g. CLSM, LSC, flow cytometry, halo-FISH, FIGE-SSGE, ChIP), we have visualized the global disassembly of the chromatin of healthy, non-apoptotic human cells and yeast protoplasts into particles apparently containing the DNA-loops. The data we have obtained provide evidence for the existence of preformed single-strand discontinuities all over the entire genome, which appear to be arranged on the two DNA strands in an alternating, staggered fashion, positioned at loop-size intervals. The chemical nature of the DNA-termini was characterized by in situ nick labeling, revealing uniform ends with 3'OH groups, tightly protected by structures sensitive to ribonucleolytic treatments. Our results suggest an association between the nicks present at the bases of chromatin loops and the nuclear matrix attached architectural hnRNA-network.

To study the role of particular DNA regions in the observed phenomena, we extended our studies on the breakpoint cluster region (bcr) of the *M*ixed *L*ineage *L*eukemia (MLL) gene that is frequently rearranged in childhood and posttherapeutic leukemias. The sequence specificity of nick accumulation and the possible role of topoisomerase II were investigated by linear primer extension footprinting and chromatin immunoprecipitation (ChIP). The results demonstrate the involvement of the above enzyme in the generation / maintenance of nicks at non-random positions. As studied by halo-FISH and ChIP, the disintegration of the chromatin at MLL bcr was dependent on local epigenetic and broader chromosomal context, with marked differences between germline and rearranged MLL. The high level of H3K-polyacetylation and H3K4-methylation at gMLL indicates transcriptionally active, relaxed chromatin structure, suggesting that the incidence of ss-breaks is highly dependent on chromosomal context. We hypothesize that the ss discontinuities present at every ~50 kbp throughout the genome might explain the frequent involvement of loop anchorage sites in chromosomal translocations.

In view of the special significance of epigenetic context in the incidence of nicks - probably predisposing for pathological gene rearrangements, we have also developed a novel high-throughput screening method for the evaluation of ChIP results, applicable in a clinical set-up, combining biophysical and molecular biological know-how.