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# Rearrangement of Chromatin Domains in Cancer and Development

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**Abstract** Both the accomplishment of developmental programs and neoplastic transformation are linked to changes in the long-range organization of chromatin, in particular, DNA loop domains. The development of new methods that allow the study of interactions between the bases of DNA loops and the proteins of the nuclear matrix will help our understanding of the molecular mechanisms in such changes. These methods should also allow the establishment of a fingerprint ''signature'' for many cancers that may serve for diagnostic purposes. J. Cell. Biochem. Suppl. 35:54–60, 2000. © 2001 Wiley-Liss, Inc.

**Key words:** nuclear matrix; DNA loop domains; development; cancer; fingerprinting

In the past, every major circus boasted its own "apeman" or "werewolf," a person with excessive hair growth on the face and body (Fig. 1). Hypertrichosis, as it is called nowadays, is an example of atavism (Lat., *atavus*, a great-grandfather's grandfather, an ancestor), the reappearance of ancestral characteristics in individuals. Atavisms illustrate how much genetic potential has been retained, although unused, during evolution. This is also true for many developmental programs that are not activated during normal development, but remain completely functional [see Hall, 1995]. Atavism is not restricted exclusively to individuals; cells within an organism can also revert to ancestral survival and developmental strategies, provoking dedifferentiation and unrestricted proliferation, in other words, cancer. Indeed, there is a striking resemblance between the behavior of cells in malignant tumors and in early embryos. In the present review we shall discuss changes that occur in long-range chromatin organization during cell differentiation and cancer.

Several levels of DNA compaction exist in eukaryotic nucleus. The DNA is packed into nucleosomes, and the resulting chromatin is further compacted into 30 nm fibres and DNA loop domains [Cook and Brazell, 1976; Paulson and Laemmli, 1977]. These loop domains can be visualized by the extraction of histones from the isolated nuclei or metaphase chromosomes where they are anchored to the proteinaceous nucleoskeleton, also called nuclear matrix or scaffold.

DNA loop size varies from 20 to 200 kbp and many genes and clusters of functionally related genes are found to be organized into distinct loops. Similarly, the organization of replicons in the genome also seems to be associated with loops as shown by co-localization of DNA loop anchorage sites with replication origins [Razin et al., 1986; van der Velden et al., 1984]. The DNA loop size also correlates with that of the replicons [Buongiorno-Nardelli et al., 1982; Marilley and Gassend-Bonnet, 1989]. DNA loops are attached to the nuclear matrix via Loop Anchorage Regions (LARs). These may include MARs (genomic elements capable of interacting in vitro in a specific fashion with isolated nuclear matrix), topoisomerase II binding sites and other sequence motifs (for a

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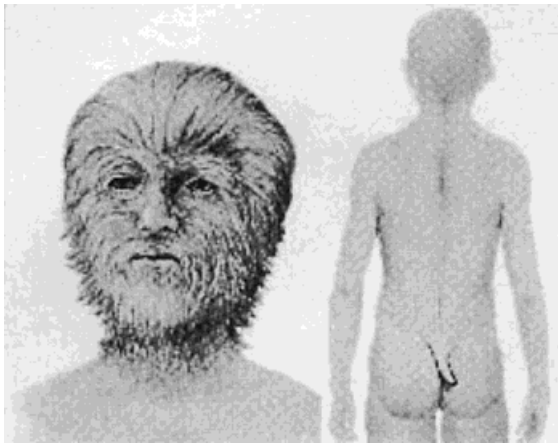

**Fig. 1.** Two examples of atavism.

review see [Razin, 1996; Vassetzky et al., 2000b]. The matrix attachment sites may be involved in development of cancer in two ways, i.e., via reorganization of the mode of genome packaging and via reorganization of the genome itself. That is why there is a good chance that in cancer cells the pattern of DNA interaction with the nuclear matrix proteins will be distinct from that typical in normal cells.

## LOOP MODIFICATION DURING DEVELOPMENT AND CANCER

A sperm pronucleus and an egg nucleus fuse after fertilization. This fusion causes a profound rearrangement of their chromatin structure. In many species, the size of DNA loops increases from ca. 50 kbp during early embryogenesis to 200 kbp in the cells of the adult organism [Buongiorno-Nardelli et al., 1982]. Metaphase chromosomes also undergo significant structural changes during development, e.g., in *Xenopus laevis* [Micheli et al., 1993].

*Xenopus* development provides an interesting model for testing transitions in gene expression during development that could be associated with changes in the organization of specific genomic domains. In *Xenopus*, the first 12 cell cycles proceed in the absence of transcription until the mid-blastula transition (MBT) when transcription is activated. We have studied the organization of DNA loop domains during development in *Xenopus laevis*. Using a conventional nuclear matrix mapping technique, we have shown that before the MBT, there are no specific attachments of the DNA loops to the nucleoskeleton. Consequent activation of zygotic transcription at the MBT has been shown in two distinct gene domains, containing rDNA and the c-*myc* genes, to be structurally associated with a specification of nuclear matrix attachment [Vassetzky et al., 2000a]. The developmental change from apparently random [Hair et al., 1998; Maric and Hyrien, 1998; Vassetzky et al., 2000a] to specific attachments of the rDNA and c-*myc* domains to the nuclear matrix may be correlated to two other transitions that occur during the same developmental period. The first is an increase in the size of chromatin loops [Buongiorno-Nardelli et al., 1982; Vassetzky et al., submitted], and the second is specification of replication origins in the same region after the MBT [Hyrien et al., 1995]. Stabilization of the rDNA chromatin domain after MBT may permit it to be structurally insulated for both transcription and replication. Significantly, after MBT both transcription and replication termination sites in the rDNA domain are located in the intergenic spacer [Meissner et al., 1991; Maric et al., 1999]. The specification of nuclear matrix attachment regions reported in this system may be involved in the establishment of stable programs of transcription during development and may contribute to the determination of stable cell lineages in the embryo.

These data are in apparent contradiction to the results obtained for *Drosophila* embryos. Association of three developmentally regulated genes to the nuclear matrix was found to be unchanged during development [Gasser and Laemmli, 1986]. However, this study was carried using re-binding of the labeled DNA to isolated nuclear matrixes. It is known that the nuclear matrix attachment sites (MARs) isolated using this method are often neither tissue- nor species-specific [Cockerill and Garrard, 1986].

It is quite possible that in vivo DNA loop organization shows cell-type specificity. Moreover, the comparison of MARs mapped using the classical in vitro MAR assay in the 800 kbp region of the *Drosophila* X chromosome [Brun et al., 1990] with the loop attachment sites revealed that in most cases, the loop borders coincided with the in vitro MARs. However, many MARs were found to be located *within* the loops [Iarovaia et al., 1996]. Hence MARs

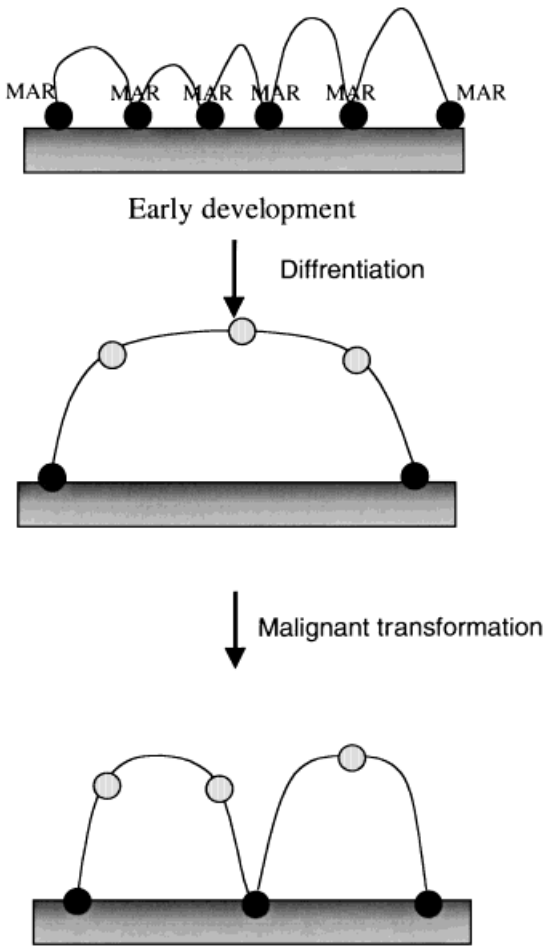


**Fig. 2.** A model of the interrelationship between the MARs and DNA loop attachment in development and cancer.

may represent potential sites of loop anchorage and that different MARs may be used to attach the DNA loops at different developmental stages or in different tissues (Fig. 2).

Another interesting implication of our findings is that DNA organization into fixed loops may serve as a security mechanism that prohibits the reversal of a developmental program. Indeed, in an early experiment on frog cloning, the direct transfer of the nucleus from a differentiated cell into the oocyte resulted in the arrest of embryogenesis at its early stages [Gurdon et al., 1975]. Successful development necessitated several passages of the nucleus in embryonic cells (Fig. 3), suggesting that a profound restructuring of the nuclear architecture is required to render the nucleus totipotent. Chromatin remodeling during nuclear transfer is an active process involving an ISWI factor. The pattern of interaction of DNA with the nuclear matrix was shown to change during the differentiation of somatic nuclei in the *Xenopus* egg cytoplasm [Kikyo et al., 2000].

Rearrangement of DNA loops was also found in transformed BHK21 cells where the average size of loops was found to decrease [Linskens et al., 1987]. Additionally, the loop size in several human cancer cell lines was found to be ca. 50 kbp, i.e., smaller than in normal cells [Oberhammer et al., 1993].

This may reflect the reversal of the differentiated state of the normal cells. Another possible explanation of this phenomenon is the structural constraint imposed by relatively rapid replication of cells in malignant tumors: replicon size is closely related to the DNA loop size and smaller and more numerous replicons mean faster replication.

## DNA TOPOISOMERASE II - MEDIATED LOOP EXCISION AND CANCER

Eukaryotic topoisomerase II is located at the bases of DNA loops in the nucleus. Exposure of mammalian cells to topoisomerase II-specific drugs that enhance its interaction with DNA has been reported to stimulate different genomic rearrangements including deletions, insertions, and translocations [Maraschin et al., 1990; Shibuya et al., 1994]. In agreement with this, it has been found that chemotherapy of tumors with topoisomerase II-specific drugs frequently causes secondary leukaemias resulting from chromosomal rearrangements [Super et al., 1993]; for review see Rowley [1993]. These rearrangements were found to occur non-randomly in the genome. The recombination points are characterized by a high degree of clustering. For example, the breakpoints of a number of translocations involving the MLL (myeloid-lymphoid-leukaemia) gene were mapped within a 8.3 kbp DNA fragment which includes preferential sites of DNA cleavage by topoisomerase II [Aplan et al., 1996]. Topoisomerase II-specific drugs also induced rearrangements in regions of the SV40 virus known to be preferentially cleaved by topoisomerase II [Bodley et al., 1993]. As shown previously, [Fernandes and Catapano, 1991; Gromova et al., 1995], topoisomerase II (i.e., the nuclear matrix-associated enzyme which interacts with DNA in loop anchorage regions) constitutes a primary target for different antitumor drugs.

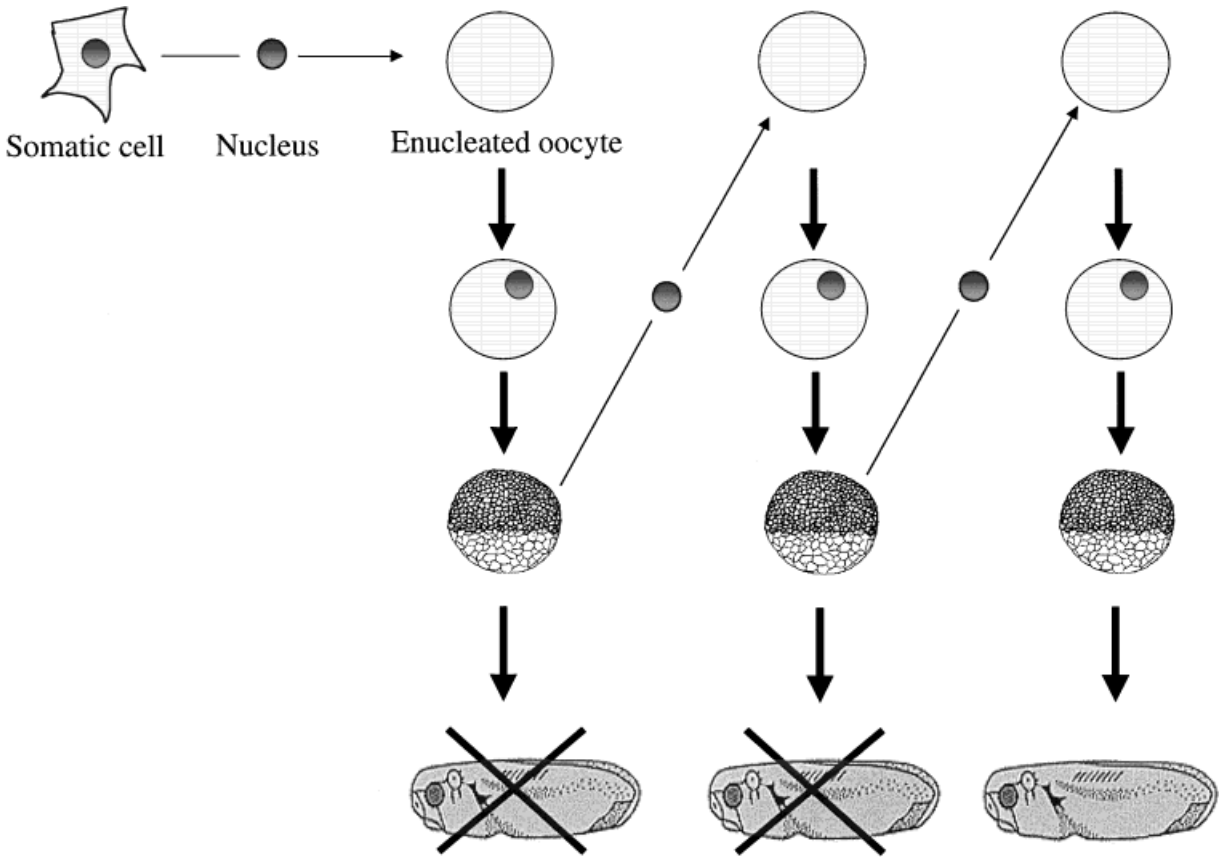


**Fig. 3.** Remodeling of the nucleus in frog cloning experiments [after Gurdon et al., 1975].

The above observations suggest that the loop anchorage regions may constitute preferential sites for illegitimate DNA recombination resulting either in the loss or in the repositioning of DNA loops. Although separated by long stretches of loop DNA, the loop anchorage regions are likely to be located close to each other in the nuclear space. This may further promote the possibility of recombination events between these regions. Indeed, as demonstrated in a model experiment, the association of circular DNA plasmids with the nuclear matrix facilitates topoisomerase II-mediated incorporation of these plasmids into catenated networks [Tsutsui and Oda, 1989].

## DNA–PROTEIN INTERACTIONS AT THE NUCLEAR MATRIX IN DEVELOPMENT AND CANCER

The anchorage of DNA loops to the nuclear matrix occurs through the protein component of the nuclear matrix. Since the organization of DNA loops is quite varied and complex, it is not surprising that neither a consensus DNA motif nor a specific protein responsible for the attachment of DNA loops to the nuclear matrix has been found (for review see [Vassetzky et al., 2000b]). At the same time several proteins that interact with the bases of the loops have been identified. Some of these proteins are found in cancer cells, while their level is relatively low in the normal cells [Bidwell et al., 1994; Yanagisawa et al., 1996; Spencer et al., 2000]. Conversely, some DNA motifs and gene fragments seem to bind specifically to the nuclear matrix in metastatic cell lines [Samuel et al., 1998; Wang et al., 1999]. Overexpression of such loop base interacting proteins may lead to rearrangement of DNA loops. In this respect, it is necessary to develop a method that would allow DNA–protein interactions at the nuclear matrix to be monitored.

To date, analysis of MARs has been restricted to fairly crude techniques for the detection of MARs by hybridization and to sequencing the DNA component of MARs [Gasser and Vassetzky, 1998]. However, we have developed a

new method based on ligation-mediated PCR (LMPCR) which, in conjunction with already well-established methods for MAR isolation [Gasser and Vassetzky, 1998], can potentially not only delineate to the nucleotide the area of DNA protein interaction in a MAR, but can also provide detail of protein–DNA interaction within the MAR itself. We have designated this method as nuclear matrix–footprinting.

This method involves the treatment of isolated permeabilized nuclei with DNaseI in a manner similar to genomic footprinting followed by the isolation of MAR-associated DNA by treatment with mild detergents or salt. The DNA component of the MAR is partially protected from the action of DNaseI while non-protein-associated DNA is destroyed. The resulting intact DNA is then isolated and used as a substrate for LMPCR.

The method of LMPCR was described in detail elsewhere [Mueller and Wold, 1989], and our method is essentially the same with some tactical differences in the positioning of the two sets of primers used for amplification and visualization. Starting from the identification of the gross region of the genome identified to contain a MAR by standard hybridization techniques, LMPCR can be used to identify to the accuracy of the individual base pair the precise limits of the DNA–matrix interaction. Two sets of primers (three primers per set) are designed in opposite orientation close to the centre of the identified MAR with the final primer of each set as close to each other as possible. These are then used in two standard PCR reactions that will generate ladders of fragments, the largest of which will mark the outermost limit of the DNA matrix interaction. No product will be generated beyond this point as DNaseI treatment destroys any non-protein-associated DNA template (Fig. 4).

With the extremities of the MAR now defined two new sets of primers can be designed as close to these points as possible oriented into the MAR. In this way and in combination with the first primer sets, the maximum possible area of the MAR can be visualized for individual protein–DNA interactions in a manner analogous to genomic footprinting. By comparison with appropriate deproteinated DNA controls areas of interaction can be seen as areas of DNaseI hypo- and hypersensitivity (see [Vassetzky et al., 2000a] for an example).

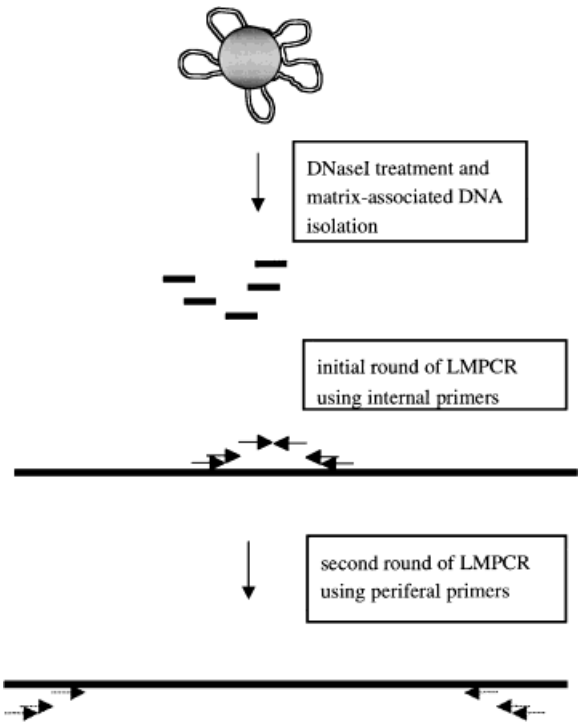


**Fig. 4.** The principle of NM-footprinting.

By using this method it will be possible to compare MARs from related normal and pathological tissues.

## THE POTENTIAL DIAGNOSTIC VALUE OF DNA–PROTEIN "SIGNATURE" IN THE CANCER CELLS

Recent advances in the study of the protein component of the nuclear matrix have allowed the characterization of several proteins that are specifically associated with the nuclear matrix in cancer cells [Konety and Getzenberg, 1999]. Some of these proteins are used for the diagnosis of cancer; e.g., NMP22 is specifically present in the nuclear matrix of bladder cancer cells [Ozen, 1999]. Hence, detecting changes in the nuclear matrix structure may serve as a valuable tool in cancer diagnostics.

It has also been shown that the pattern of interaction of specific DNA sequences with the nuclear matrix is altered in the tumor cells [Samuel et al., 1998; Wang et al., 1999]. This finding along with the data on changes in the long-range organization of the DNA loops provide an interesting possibility of identifying "signatures" of different cancers at the DNA–matrix interaction level. A tissue sample could be processed using nuclear matrix-footprinting.

The changes at the loop bases detected using this method may have potential use in cancer diagnostics. One of the potential regions of interest is the breakpoints region in the MLL gene [Aplan et al., 1996]. One can also envisage a systematic study of known LARs in normal vs. tumor cells in order to detect changes in the loop organization linked to malignant transformation. Since the NM-footprinting is a PCR-based assay, it can be used on small amounts of material, in the same way as DNA fingerprinting is used for the detection of specific DNA within minimal amounts of starting material. Further studies using NM-footprinting may also provide precious information on the nature of DNA–protein interactions within the LARs and allow the identification of new specific proteins that mediate organization of the genome into functional loop domains in normal and transformed cells.

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