Scaffold/Matrix Attachment Regions (S/MARs): Relevance for Disease and Therapy

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Abstract There is increasing awareness that processes, such as development, aging and cancer, are governed, to a considerable extent, by epigenetic processes, such as DNA and histone modifications. The sites of these modifications in turn reflect their position and role in the nuclear architecture. Since epigenetic changes are easier to reverse than mutations, drugs that remove or add the chemical tags are at the forefront of research for the treatment of cancerous and inflammatory diseases. This review will use selected examples to develop a unified view that might assist the systematic development of novel therapeutic regimens.

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1 Introduction

Despite the rapid progress in sequencing eukaryotic genomes, our current abilities to interpret sequence information are still limited. Progress is expected from knowledge about the functional links between nuclear architecture and gene expression patterns on one hand and the establishment and maintenance of expression patterns during development on the other. Along these lines, the principles are explored that account for the compartmentalization of replication and transcription machineries within the nuclear compartments. These compartments assemble factors to an extent enabling protein–protein and protein-DNA interactions, and they serve the integration of regulatory signals into specific signal transduction pathways.

During recent years, epigenetic, chromatin-activating principles have entered the stage (review: Hake et al. 2004) together with the elements that delimit differentially regulated domains, so-called genomic insulators and/or boundary elements (Goetze et al. 2005). New evidence has emerged to help understand the role of chromosome territories (CTs), i.e. the structural equivalent of metaphase chromosomes at interphase, together with the interchromatin domain compartment (ICD), originally interpreted as a chromatin-free channel system in between the CTs. Ultimately, such a simplistic model was not consistent with the high frequency of complex chromosomal aberrations, which indicated the presence of inter-chromosome contacts within this space (Bode et al. 2000a; Branco and Pombo 2006).

When Kanda et al. (1998) stained the entire chromatin compartment in living cells, using histone H2b-GFP fusions they could localize putative factor storage sites, such as speckles, Cajal bodies and PML bodies, to extended portions of the interchromatin space, and they demonstrated that chromatin loops can in fact expand into this compartment. This led to the view that active genes interact with the transcriptional machinery only if they are positioned at the surface of CTs or on its looped extensions. Transcriptionally "potentiated" (otherwise called "poised") genes such as the quiescent, but inducible type-I interferon genes were found in a related position (Winkelmann, 2007). Quiescent genes on the other hand were thought to reside within the CTs. This model had to be refined once more when transcription and splicing could not only be observed at the periphery, but also appeared to extend into the territories. This was later ascribed to a highly folded CT structure that still permits access to certain genes that line this interior ICD-channel system (Albiez et al. 2006, Branco and Pombo 2006).

The question if a polymer meshwork, a so-called "nuclear matrix" or "nuclear scaffold", is an essential component of the in vivo nuclear architecture is still a matter of debate (Martelli et al. 2002). While there are arguments that the relative position of CTs may be maintained due to steric hindrance or electrostatic repulsion forces between the apparently highly structured CT surfaces, such an idea has to be reconciled with the following pilot observations:

- When Maniotis and colleagues (1997) "harpooned" nuclei, they could pull out all of the nucleoplasm on a string in interphase and all the chromosomes on a string in metaphase. Depending on the presence of Mg^{++}, they observed unwinding and rewinding of these structures. This effect was lost upon mild DNAse treatment, indicating that the structure of DNA and its associated scaffolds are responsible for this phenomenon;
- Ma et al. (1999) treated cells in situ with the classic extraction procedures that are otherwise used to isolate the nuclear matrix. Chromosome-painting techniques clearly demonstrated that territories remained intact up to the point where a minor subset of acidic nuclear matrix proteins was released – potentially those proteins that governed their association with a nuclear skeleton.

The existence of a nuclear skeleton was first proposed about 60 years ago (Zbarsky and Debov 1948), and methods for the preparation of such an entity have been developed and refined ever since (reviewed by Martelli et al. 1996). We will maintain the idea that a nuclear skeleton acts as a dynamic support for many specialized reactions as the most suggestive model to guide the reader through this review. This concept will rationalize current efforts that are dedicated to inhibitors affecting the interaction of distinct transcription factors with the protein components of such a matrix and its associated DNA elements.

After a brief overview of the architectural principles of eukaryotic genomes, our discussion will deal with the properties of certain DNA regions that can serve as scaffold/matrix attachment regions (S/MARs), which are DNA elements with a well-established spectrum of biological activities. In this context, we will address the dynamic properties of prominent constitutive and facultative fibre-forming protein scaffold constituents and continue with factors that associate with the relevant protein or DNA interaction partners. Regarding the first class, we emphasize the lamins and hnRNPs and their functional interactions. For the second class, we will focus our attention on those examples that hold promise to either assist diagnosis or to lead to administrable pharmaceuticals. Here the ubiquitous poly(ADP-ribosyl) polymerase (PARP-1) and the cell-specific factor SATB1 will serve as the cores within networks of multiple interacting factors with a relation to the scaffold, to S/MARs or to both. It is anticipated that these paradigms will strengthen work at the verge of in vivo and in vitro studies all the more as these projects can now be guided and coordinated by up-to-date system biology approaches (examples are the inserts in Figs. 1 and 2).

1.1 Relevance of Non-Coding DNA: "Junk-DNA" and Gene Deserts

The idea that the amount of DNA per chromosome set might be constant for all cells within individuals appeared more than a century ago. In 1948, Vendrely and Vendrely could confirm this assumption, and they defined the "C-value," the nuclear DNA content per cell, in all the individuals within a given species. These observations provided the first clue that DNA rather than protein is the heritable material.

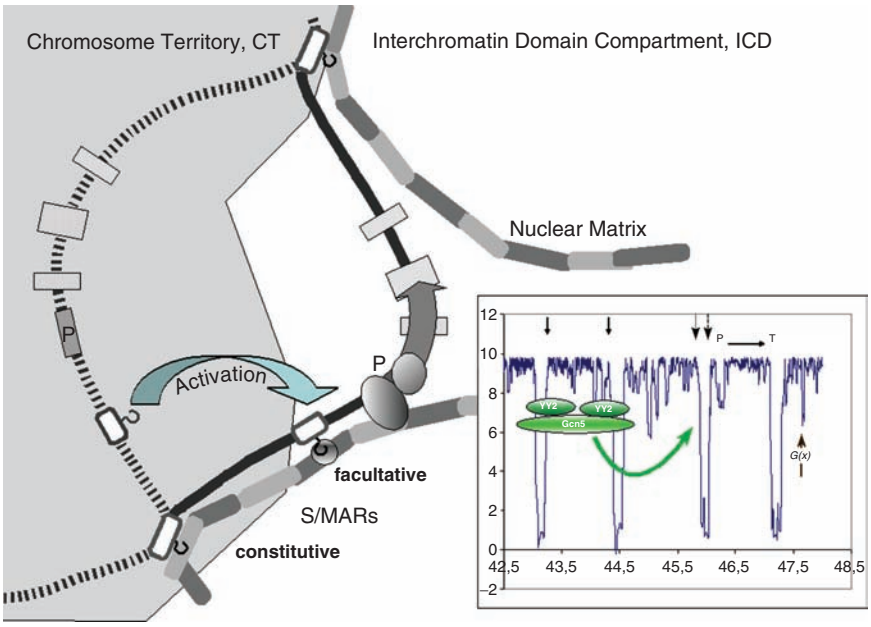

Fig. 1 S/MARs in the framework of the CT-ICD model. The eukaryotic genome is organized into chromatin domains, each of which is delimited by an extended "constitutive" S/MAR, i.e. an element that is permanently attached to components of the nuclear matrix; the matrix itself fills major parts of the ICD compartment. The assembly of activating/remodeling factors at short domain-internal scaffold/matrix attachment regions accompanies gene activation. Thereby these "facultative" S/MARs mediate the factor-induced (reversible) association with the matrix. SIDD profiles can efficiently assist the classification of these elements (reviewed by Winkelmann et al. 2006): while a constitutive S/MAR consists of a series of evenly spaced "unpairing elements" (*UEs*; minima in the SIDD profile), which together form a "base-unpairing region" (*BUR*), the "facultative" class mostly consists of 200–300-bp-long strongly destabilized individual UEs that are separated by >500 bp (see text and Bode et al. 2006). The *insert* exemplifies the latter situation: for the human interferon-β gene domain, all UEs (i.e. the four pronounced minima) coincide with DNAse I hypersensitive sites and do have regulatory potential. Two of these elements associate, each with a molecule of YY1/YY2 (*small elliptic bodies*), which in turn recruit a histone-acetyltransferase molecule (*extended ellipse*) to support activation of the inducible promoter (Klar and Bode 2005). The outline of this figure follows discussions with Thomas Werner (Genomatix Munich) and comprises the concepts by Bode and colleagues (2003a,b)

Soon it was found, however, that genome sizes vary enormously among eukaryotes and that size bears no relationship to the presumed number of genes (the so-called "C-value paradox"; Thomas 1971): while one copy of a human's genome contains about 3.5 pg of DNA packaged into 23 chromosomes, the 5.8-pg equivalent of an aardvark genome is contained in only ten chromosomes and the 140 pg in the genome of some salamanders in only 12 chromosomes. Triggered by the question whether eukaryotes evolved large genomes simply because they can tolerate useless DNA or because they need them for organization or function, the view that transcriptional regulation operates at the level of individual genes had to be continuously extended.

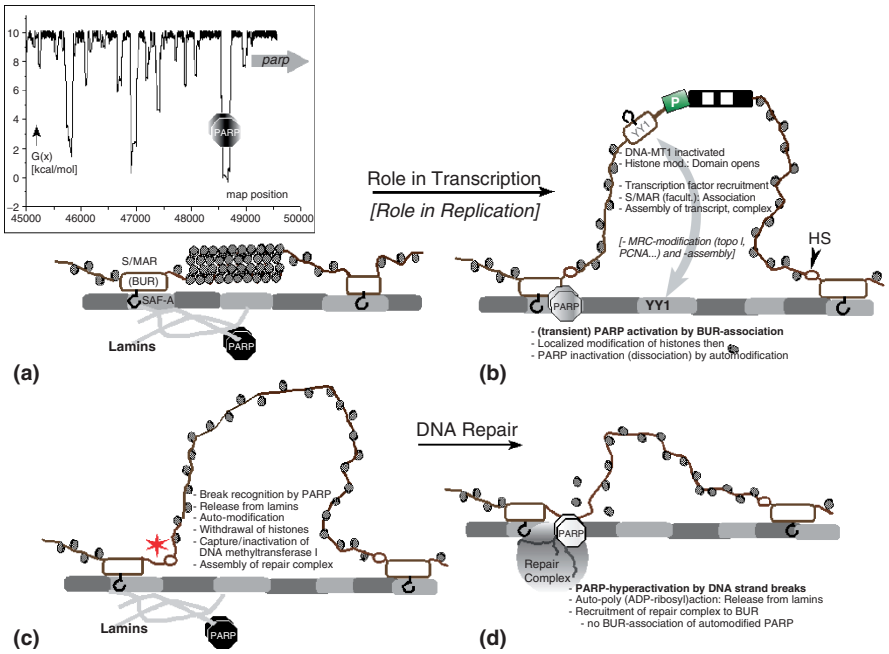

Fig. 2 Functional states of PARP-1. Inactive forms have been drawn in *black*, and increasing activity is indicated in *grey* or *light-grey colours*, respectively. The *insert* (SIDD profile, **a** analyses the PARP promoter for the presence of S/MAR-like elements, mediating the gene's autoregulation (Soldatenkov, 2002). Under certain circumstances, PARP association with an S/MAR may induce activity and enable PARP to transactivate certain genes due to a variety of domain-opening functions (Lonskaya et al. 2006); the figure (**b**) comprises both constitutive elements and a facultative S/MAR element as defined in Fig. 1. PARP-1 has also been shown to be a component of the multiprotein DNA replication complex (*MRC*); it poly(ADP-ribosyl)ates 15 of the ~40 MRC proteins, including DNA pol α, topo I and PCNA. Note the DNAse I hypersensitive site (*HS*), which is a frequent concomitant of constitutive domain borders (Sect. 3)

To account for these developments, we tend not to talk about "genes" anymore, but prefer the terms "chromatin domains" or, more generally, "transcriptional units" for any autonomous regulatory entity in the genome.

For decades, geneticists have focused on just those 2% of mammalian DNA that contain blueprints for proteins, while the remainder was sometimes dismissed as "junk". Actual scans of the mouse genome led to estimates according to which there are between 70,000 and 100,000 transcriptional units, half of which are non-coding. The discovery of these "hidden genes", which work through RNA rather than protein, has initiated a re-thinking, the more so as active forms of RNA are now known to provide an additional level of regulation. Nowadays, long genomic regions without any obvious biological function are referred to as "gene deserts" (Venter et al. 2001). Again, for some of these deserts regulatory sequences could be localized that exert control functions over large distances (Nobrega et al. 2003). Many of these

units have particular evolutionary histories and sequence signatures that make them distinct from the rest of the genome. Other gene-sparse regions, however, may in fact be nonessential to genome function, since they could be deleted without significant phenotypic effects (Nobrega et al. 2004). Information of this kind will be essential for researchers looking for mutations causing disease, because it highlights large areas of the genome that are unlikely to be involved in such a process.

In the context of such a classification, it appears rewarding to consider the genomic distribution of retroelements. Retroelements are involved in shaping the genome and have guided its evolution, extension and organization. Besides endogenous retroviruses, there are populations of truncated retroelements, such as the long, interspersed nuclear elements (LINEs), which constitute about 5% of the total human genome and may encode a functional reverse transcriptase. The short, interspersed nuclear elements (SINEs) represent an even larger proportion, but have many deletions. SINEs can modulate gene expression by movement, amplification and re-insertion into genes and regulatory sequences, but to do so they have to depend on reverse transcriptase from other sources. The human prototype SINE, the Alu repeat, is roughly 300 nucleotides in length. An RNA polymerase III start site is located within some repeats that can direct transcription in response to viral infections or the exposure to carcinogens. This expression may facilitate recombination with other Alus or their flanking regions, and this may be one reason for the fact that Alu sequences are frequent concomitants of chromosomal breakpoints. While LINEs tend to be found in AT-rich DNA, characteristic of intergenic regions, SINEs, and Alus in particular, are more often located in GC-rich regions, where genes tend to reside. This location does not seem to be a function of insertion site preference, but rather appears to be due to differential retention principles. In this respect, it is of note that SINEs participate in the transcriptional regulation of certain genes, suggesting a continuous selection against their random accumulation.

During evolution, retrotransposons have steadily screened mammalian genomes for the most attractive integration sites. For a deeper understanding, we have to consider the nature of these preferred sites. Our studies have clearly demonstrated that, without an exception, all provirus integration sites are associated with a S/MAR (Goetze et al. 2003b; Johnson and Levy 2005 and references therein). The integration of retrotransposons may obey the same rules that govern retroviral integration, and therefore the location of these elements may simply represent a marker for the presence of S/MARs. How then are S/MARs distributed over the genome? A study by Glazko et al. (2003) has localized numerous homologous intergenic tracts (HITs) of largely unknown function in orthologous human-mouse genomic regions. Fifty percent of the hits could be correlated with predicted S/MARs, which suggests that these conserved elements have probably retained their function during the 80–100 million years since the radiation from their common ancestor. The other half of predicted S/MARs turned out to be non-conserved. This group is hence likely to be species-specific and might mediate unique functions. Interestingly, an excess of orthologous S/MARs was observed in spacers between divergently transcribed genes, while there were no conserved S/MARs located between convergent genes. This distribution suggests that the conserved elements are primarily involved in the

regulation (augmentation) of transcription initiation, which would be in accord with a study from this laboratory (Schuebeler et al.1996).

2 Scaffold/Matrix Attachment Regions (S/MARs): DNA at the Scaffold

Obviously, S/MARs map to non-random locations in the genome. They occur at the flanks of transcribed regions, in 5′-introns and telomeres, and also at gene breakpoint cluster regions (review: Bode et al. 1995). S/MARs are association points for common nuclear structural proteins (review: Bode et al. 2000b and below) and proved to be required for authentic and efficient chromosomal replication and transcription, for recombination and chromosome condensation. These are the levels of their firmly established biological activities:

- *Transcriptional level*
  - S/MARs augment gene expression by increasing transcription initiation rates; they are not active in transient expression systems as they require incorporation into authentic (replicated) chromatin structures (review: Bode et al. 1995).
  - They provide long-term stability as they contribute to the assembly of the histone acetylation apparatus (review: Bode et al. 2003).
- *Transcriptional competence*
  - S/MARs either cooperate with genomic insulators or they function as insulators themselves (Antes et al. 2001; Goetze et al. 2005).
  - They enable the topological separation of independently regulated transcription units (Bode et al. 1992; review: Bode et al.1996).
- *Origin-of-replication (ORI) support*
  - Eukaryotic ORIs are consistently associated with S/MAR elements (review: Bode et al. 2001) where these provide ARS-like functions not only in yeast (AK and Benham 2005), but also in mammalia (Nehlsen et al. 2006).
- *Recombination hotspots*
  - S/MAR-associated DNA structures are involved in the generation of breakpoint-cluster regions (BCRs; review Bode et al. 2000a). They also guide the integration of retroviral genomes (Mielke et al. 1996).

There are more recent indications for an additional role in interphase chromatid cohesion and/or separation (Mesner et al. 2003). S/MARs do not have an obvious consensus sequence. Although prototype elements consist of AT-rich regions several hundred base pairs in length, the overall base composition is definitely not the primary determinant of their activity. Instead, binding and (biological) activity appears to require a pattern of "AT patches" that confer the propensity for local strand unpairing under torsional strain (Bode et al. 2006). Both chemical and enzymatic probes have originally been applied to show that this strand separation potential is

utilized in the living cell for anchoring a chromatin domain to the matrix and that DNA accessibility is modulated at times of transcriptional activity (review: Bode 1995). Subsequent bioinformatic approaches support the idea that, by these properties, S/MARs not only topologically separate each domain from its neighbours (Bode et al. 1992), but also provide platforms for the assembly of factors supporting transcriptional events within a given domain (Bode et al. 2003b).

The strand separation potential of a S/MAR is commonly displayed in the form of a stress-induced duplex destabilization (SIDD) profile, which predicts the free energy $G(x)$ needed to effect separation of the base pair at each position x along the DNA sequence, at a certain level of torsional tension (review: Winkelmann et al. 2006). The energy stored in a base-unpaired region (BUR) can serve the formation of nearby cruciforms or slippage structures. These alternate structures, as well as single-stranded bubbles, are recognizable features for DNAses, topoisomerases, poly(ADP-ribosyl) polymerases and related enzymes (see below).

Originally, matrix-attachment elements (MARs) were characterized by their specific (re-)association with the nuclear matrix (i.e. the remnants of a salt-extraction protocol), whereas scaffold-attachment elements (SARs) were mostly characterized by their (re-)association with nuclear scaffolds [i.e. the remnants of a lithium 3,5-diiodosalycilate (LIS, a mild detergent) extraction procedure in the presence of a vast excess of bacterial competitor DNA; Kay and Bode 1995]. The observation that the elements recovered by the reassociation methods are identical or closely related has led to the consensus-term "S/MAR". Moreover, the outcome of the LIS procedure does not depend on the source of the nuclear scaffolds, as there is cross-competition between S/MARs from plants and mammals (Mielke et al. 1990), and it can be simulated by computer-assisted routines.

The binding of various forms of DNA to the nuclear scaffold has been extensively characterized (Kay and Bode 1994). As a whole, the scaffold has a strong tendency to bind single-stranded (ss) as well as supercoiled (sc) DNA. Recognition of scDNA has been ascribed to topoisomerases, since LIS-extracted scaffolds retain a pronounced nicking-closing activity. This activity appears to occur at a distinct subset of sites, as externally added S/MAR sequences and ssDNA do not interfere with the process. In contrast, there is a competition between ssDNA binding and prototype S/MAR binding on some scaffold-associated proteins, but not on others (Kay and Bode 1994; Mielke et al. 1996). In retrospect, competition patterns have proven valuable as they can be applied to reveal specific binding modes. This criterion, for example, has served to identify a novel class of S/MARs in an extended non-coding region where we detected a striking periodicity of narrow SIDD minima, which obey a periodicity of roughly 2,500 bp (Goetze et al. 2003a). A functional comparison revealed that these elements, in contrast to prototype S/MARs, have transcriptional augmentation, but no insulation activity, hinting at the existence of distinguishable classes of S/MARs (Goetze et al. 2005). While the uniform register of these elements might indicate an involvement in upper levels of chromatin organization, it is also possible that these signals serve regulatory functions, for instance in the expression of transcripts of unknown function (TUFs) that are present across large sections of the human genome.

S/MARs have been classified as either being constitutive (demarcating permanent domain boundaries in all cell types) or facultative (cell type- and activity-related) depending on their dynamic properties (details in Fig. 1 and Sect. 3.2). In the first case, the elements are marked by a constitutive DNAse I hypersensitive site in all tissues (Bode et al. 1995), which typically coincides with the preferred cleavage sites for endogenous topoisomerase II (topo II) in living cells (Iarovaia et al.1995). In the second case, hypersensitivity is correlated with either the potentiated state or active transcription (Heng et al. 2004). S/MARs partition the genome into 50–200-kb regions demarcating chromosomal (sub-)domains and/or replicons, and the number of elements that attach the ends of a (sub-)loop to the scaffold approximates 64,000 (see Fig. 1). There are likely an additional 10,000 S/MARs supporting replication foci. Apart from the technique used to derive such a conclusion, the time of observation and the cell type will also determine what subset of the total number of the estimated 74,000 S/MARs is detected (Linnemann et al. 2007). In 2006 still only a minor fraction of S/MARs (i.e. 559 for all eukaryotes) had met the standard criteria for an annotation in the S/MARt database (Liebich et al. 2000; http://sS/MARtdb.bioinf.med.uni-goettingen.de/).

Figure 1 summarizes the criteria by which the nuclear matrix/scaffold can be functionally integrated into the CT/ICD architecture.

2.1 S/MAR Aberrations and Disease

Changes in nuclear matrix attachment by either the loss of association or by binding to a previously cryptic site have been implicated in the onset of several genetic diseases and disorders. In case of male infertility, the loss of a S/MAR was found to arrest expression (Kramer et al. 1997). On the other hand, interactions between originally "domesticated" elements may trigger genomic instability such as for various forms of cancer where sites of chromosomal fragmentation localize to S/MARs (review: Bode et al. 2000). Although some of the mechanisms remain unknown, S/MARs continue to reveal a multitude of varied roles in pathogenesis that reflect aspects of their complex nature (Linnemann et al. 2007; review: Galande 2002).

Nowadays high-throughput technologies provide the opportunity to directly determine the distribution of scaffold/matrix attachment points in situ across an entire eukaryotic genome. While the first genomic arrays were based on BACs, their resolution could be increased by the use of cosmids and now of oligonucleotides. These tools are being applied to identify endogenous human S/MARs from chromosome 16 in preparations that employ nuclear extraction with either 25 mM or 2 M NaCl, which leaves the nuclear matrix DNA compact, while the non-matrix DNA forms a surrounding halo (Goetze et al. 2003b). Restriction nuclease digestion releases the loop DNA from the matrix, enabling the separation by centrifugation of matrix-associated DNA (pellet) and loop DNA (supernatant). Data for chromosome 16 highlight a variety of differential characteristics for the loop and matrix-bound portions: loop regions are relatively widespread in contrast to the discrete highly

dense regions of matrix attachment. As expected, the tightly grouped matrix-associated regions are mostly observed in gene-rich regions within intronic GC rich segments, whereas a smaller fraction localizes to the borders of extended co-regulated transcription units (Linnemann et al. 2007).

A combination of traditional fractionation protocols, combined with array and PCR technologies, will both refine and accelerate the identification of S/MARs in the human genome. It has been proposed that two of the prevailing strategies, the LIS- and NaCl-based in situ extraction procedures, recover different types of S/MARs: whereas LIS is known to disrupt transcription complexes, NaCl has been shown to disrupt replication *foci*. Accordingly, the procedures recover distinct groups of S/MARs associated with either transcriptionally inactive or active regions (Linnemann et al. 2007). Such a genome-wide identification will enable understanding the mechanistic role of S/MARs in disease and development. An example is the recent report by Petrov et al. (2006), who demonstrate a functional link between the de-localization of a defective chromosome segment from the nuclear matrix and malignant changes in gene expression. These studies underline the predictive value of identifying S/MARs in situ by the "association-approach" (Sect. 3), the more so as the results can be substantiated by a haloFISH method.

2.2 BURs as Targets for Anticancer Therapy

SIDD profiles (i.e. plots of G versus map position) are potent predictive tools for localizing "base-unpairing regions" (BURs), which are the hallmarks of S/MARs. BURs either consist of one dominant $G(x)$ minimum exceeding a threshold extension of 200–300 bp (see inserts to Figs. 1 and 2) or of a succession of multiple, evenly spaced, but moderately destabilized "unpairing elements" (UEs). In the latter case, if spacing between restricted UEs exceeds ≈500 bp, individual elements lose their capacity to communicate with each other, concomitant with a loss of scaffold-binding activity (Bode et al. 2006). In any event, part or all of the BUR would become single stranded at sufficient superhelicity. The relevance of these features is underlined by the success of BUR-affinity chromatographic separation procedures, which served to isolate and identify a considerable number of S/MAR-associated proteins (review: Galande et al. 2002), among these ubiquitous representatives such as PARP-1, Ku autoantigen, HMG-I(Y), nucleolin, mutant p53 and cell-type specific factors such as SATB1 (T-cells) and BRIGHT (B-cells). The observation that prototype BUR binders are early targets for a caspase-mediated apoptotic cleavage is of particular present interest. Among these, PARP-1 and SAF-A/hnRNP-U are cleaved simultaneously by caspase 3, whereas SATB1 is uniquely cleaved by caspase 6.

BURs have recently emerged as general targets for cancer therapy, most likely since distinct BUR-binding proteins are up-regulated in carcinomas (Galande et al. 2002). The ability of small molecules to target regions with BUR potential may therefore provide a general approach for counteracting deleterious effects in

these regions. First of all, the naturally occurring oligopeptide distamycin A prefers A/T-rich DNA sequences for binding to the minor groove of double-stranded DNA, whereby strand unpairing and the association of BUR-specific factors is prevented. This principle can be fortified by using distamycin derivatives to carry cytotoxic alkylating moieties into these regions. On the other hand, there are a number of small molecules available to target BUR-type sequences directly. This group comprises two DNA-specific antitumour agents of the cyclopropylpyrroleindole family, bizelesin and adozelesin (Schwartz et al. 2003), which share a high specificity for AT-patches/BURs. Bizelesin has the highest potential for conferring regiospecific alkylation and is one of the most cytotoxic compounds ever identified: it produces a significantly higher number of lesions in several prominent AT-rich islands within cancer cells than in the bulk of genomic DNA. Strikingly, the SIDD-derived S/MAR potential (Sect. 3 and Winkelmann et al. 2006) of the affected regions correlates well with the total number of bizelesin sites (Woynarowski et al. 2001). Presently the design, chemical synthesis and in vitro testing of BUR-specific polyamides and other cyclopropylpyrroleindole-derived small molecules are underway in order to exploit their highly effective anti-tumor potential.

So far, we have more or less considered the global properties of BUR-binding proteins. If we add, as another criterion, the class of S/MARs they interact with, the following subdivision emerges:

- Constitutive contacts: mostly established by extended S/MARs at the borders of a chromatin domain by association with ubiquitous scaffold proteins, such as scaffold attachment factor A (SAF-A, otherwise known as hnRNP-U), the lamins and possibly NuMA/actin. These "bordering elements" are accompanied by DNAse hypersensitive sites in all cell types
    - Constitutive matrix proteins bind according to the mass-binding phenomenon in a cooperative process. Series of binding sub-sites (i.e. of multiple UEs, which together form a BUR) may correspond to "AT patches," "AT hooks," "SAF boxes" or their equivalents (see Sec. 5.2 and Fackelmayer 2004).
    - Constitutive S/MARs are platforms for the assembly of chromatin-modulating proteins such as topo II and related enzymes, which at these sites initiate apoptotic degradation, histone-acetyltransferases/–deacetylases and methyl-binding proteins such as MeCP2/ARBP (Straetling and Yu 1999).
- Facultative S/MARs: more restricted, domain-internal sites, which in the extreme case consist of a single UE (insert to Fig. 1). These elements preferentially associate with tissue-specific proteins
    - These proteins may be rare transcription factors, which are concentrated at the scaffold owing to a nuclear matrix-targeting sequence (NMTS; Zeng et al. 1997) or due to their association with a central player such as PARP, which exhibits many activity-dependent modes of binding (Sect. 5.1).
    - Cell-type specific factors (such as SATB1 in T-cells, SATB2 and BRIGHT in B-cells) that establish a dynamic equilibrium within the domain (see Sect. 5.2 and the dynamic model depicted in Fig. 1).

The dynamic properties of nuclear matrix functions may explain why this scheme must not be considered a rigid one and why we have to expect factors commuting between both groups.

3 Scaffolding: Ubiquitous Fibre-Forming Components and Their Associated Functions

The backbone of eukaryotic nuclei can be isolated and characterized according to protocols that have been optimized for the removal of soluble proteins, such as histones. For instance, the scaffolds resisting LIS extraction contain components from three nuclear compartments, (1) the lamina (lamins A-C), (2) the nucleolus (nucleolin) and (3) the fibrogranular internal network intermediate filament (IF-type proteins). Ludérus et al. (1992, 1994) have studied the distribution of S/MAR binding centres over the scaffold and found attachment sites distributed equally over the peripheral nuclear lamina and the internal fibrogranular network. The domains observed in nuclear matrix preparations by confocal or electron microscopy are neither collapsed nor floating free. They are rather retained in a precise spatial relationship to other landmarks, demonstrating the function of such a supporting structure. The core of these non-chromatin nuclear structures is formed by the fibrogranular ribonucleoprotein (RNP) network together with two networks of intermediate filaments that stabilize the nuclear envelope from both the outside and the inside. On the outside, a cocoon of filaments connects the nucleus with the cytoskeleton, while the inner face of the nuclear membrane and the nucleus' interior is reinforced by lamins. In combination with these networks, S/MARs impart a wide spectrum of functional properties as they restrict genomic areas to particular nuclear compartments.

3.1 Lamin Networks

Lamin proteins are type-V IF proteins that, unlike other family members, assemble to branched filaments. Besides the cage-like framework at the inner nuclear membrane, lamins are also found throughout the nucleus: fusions of lamins A and B with GFP reveal a homogenous nucleoplasmic "veil" in addition to the intensely fluorescing nuclear lamina. Mobility measurements show that within the veil lamins are more mobile and somewhat less resistant to the conventional extraction steps. Jackson (2005) has described a branched intermediate filament network that ramifies throughout the nucleus of human cells. RNAi techniques were applied to displace each of the nuclear lamin proteins from the filaments with the consequence that the structural changes correlated with profound effects on both RNA and DNA synthesis. An almost complete cessation of transcription by RNA polymerase II and an approximately 70% decrease in the number of S-phase cells suggest that lamin networks contribute to the regulation of chromatin function.

Metazoan cells express A- and B-type lamins, which differ in their length and pI value. While B-type lamins are present in every cell, A-type lamins are only expressed following gastrulation. In humans there are three differentially regulated genes: Lamin A and C are splice variants of the LMNA gene found at 1q21, whereas lamins B1 and B2 are expressed from the LMNB1 and LMNB2 genes on 5q23 and 19q13, respectively. Lamins B (and later A) have been determined as major S/MAR-binding partners within the nuclear scaffold. The relevant binding properties could be reproduced with paracrystal-like lamin polymers revealing two activity-dependent modes that appear to be related to different features of S/MARs. One type involves the regions with single-strand potential and the other the minor groove of the DNA double strand. Both modes of association are interdependent as S/MAR binding is almost completely inhibited by the presence of single-stranded competitors.

3.1.1 Laminopathies

The recent discoveries that mutated lamins and lamin-binding nuclear membrane proteins can be linked to numerous rare human diseases (laminopathies) have changed the cell biologist's view of lamins as mere structural nuclear scaffold proteins (review: Zastrow et al. 2004). So far, however, we can only speculate why mutations in lamin A/C or in the associated emerin or the lamin B receptor genes result in such a wide range of tissue-specific phenotypes and how different mutations in the same gene can give rise to such a diverse set of diseases: Emery-Dreifuss/limb girdle muscular dystrophies, dilated cardiomyopathy (DCM), familial partial lipodystrophy (FPLD), autosomal recessive axonal neuropathy (Charcot-S/MARie-Tooth disorder, CMT2), mandibuloacral dysplasia (MAD), Hutchison Gilford Progeria syndrome (HGS), Greenberg skeletal dysplasia and Pelger-Huet anomaly (PHA). All the mentioned matrix constituents are known to interact with DNA and/or chromosomal proteins, including the core histones, and they provide a complex dynamic link between the peripheral lamina and nucleoskeletal structures. It is anticipated that understanding the cellular dysfunctions that lead to laminopathies will further enhance our insight into the specific roles of the lamina in nuclear organization (review: Burke and Stewart 2002, 2006).

A particular group of LMNA mutations leads to a progeroid disease called "atypical Werner's syndrome" (WS). Fibroblasts from affected patients show a substantially enhanced proportion of nuclei with altered morphology and a disordered lamin structure. So far, there is no molecular explanation for a progeroid disease associated with lamin functions. However, a clue may arise from relating this atypical form to the prototype WS, an inherited disease characterized by sensitivity to DNA-damaging agents, by genomic instability and premature aging: Prototype WS is caused by a missense mutation in the gene of a RecQ family helicase/exonuclease (WRN) for which one of the postulated functions is the participation in a replication complex (Chen et al. 2003). For WS cells the poly(ADP-ribosyl)ation of cellular proteins is severely impaired, suggesting a relation between WRN and PARP-1. Immunoprecipitation studies and protein interaction assays in fact indicate

direct association of PARP-1 with WRN and the assembly of a complex together with Ku70/80. In the presence of DNA and NAD^+, PARP-1 modifies Ku70/80, but not WRN, and it undergoes the typical automodification reaction. These events reduce the DNA binding capacity of Ku70/80 and its potential to stimulate WRN activity, demonstrating that PARP-1 is definitely involved in its regulation (Li et al. 2004). A report by Vidakovíc et al. (2004) proving that the association with lamins modulates the activity of PARP-1 may provide the missing link between the two forms of the syndrome (atypical and prototype; cf. Fig. 2).

Poly(ADP-ribosyl)ation has frequently been linked to longevity, as differences in the catalytic activity of PARP-1 closely correlate with differences in life span. These findings together suggest a functional link between WRN, PARP-1 and Ku70/80, which can consequently be considered as caretakers of genome integrity.

3.2 hnRNPs: SAF-A

Mattern et al. (1997 and references therein) have identified the most abundant proteins that are exclusively present in the internal nuclear network. In line with earlier reports (Nakayasu and Berezney 1991), many of these belong to the group of heterogeneous nuclear ribonucleoproteins (hnRNPs), the sites of nascent transcripts and RNA maturation. These findings support a model in which major matrix protein constituents are involved in RNA metabolism, packaging and transport.

The most abundant component of this group, called "scaffold-attachment factor A" (SAF-A/ hnRNP-U), was first characterized in LIS-extracted scaffolds (Kipp et al. 2000 and references therein). SAF-A associates with multiple S/MARs, and UV-cross-linking experiments show that this established RNA binder is also associated with DNA in vivo. HeLa-cells contain about 2 million molecules per nucleus, half of which associate with the nuclear matrix in a salt-resistant manner. The other half is either bound to hnRNP particles or resides in a DNAseI extractable fraction.

The primary structure of SAF-A reflects its dual function as there are two independent nucleic acid-binding domains, (1) a C-terminal RNA/ssDNA binding domain (RGG box) and (2) a S/MAR-specific 45-amino acid N-terminal domain, called "SAF box", which is split and inactivated during apoptosis (Kipp et al. 2000). The SAF box is reminiscent of a homeobox lacking the DNA recognition helix and was the first characterized protein domain specifically recognizing S/MARs. SAF boxes are present in organisms as distant as yeast, plants and mammals, but not in prokaryotes, compatible with their specific binding to S/MAR-DNA. Originally unexpected, SAF-box-containing proteins from evolutionarily distant eukaryotes are not orthologs since homologies outside the SAF box are barely detectable. For most of these proteins, the function has remained unknown, with the exception of SAF-B and E1B-AP5, which serve related functions in the nuclear architecture and/or RNA metabolism. Interestingly, a poly(ADP-ribosyl)ase (PARP) from *A. thaliana* contains two SAF boxes in tandem, suggesting that these can substitute for certain functions of, for instance, Zn-finger domains (Kipp et al. 2000). Being a ubiquitous S/MAR-binding

component of the nuclear matrix, a separate chapter Sect. 5.1) will be devoted to PARP-1, the major representative of this class in mammals.

In vitro, SAF-A shows a pronounced propensity to self-polymerize, and this state is required to recognize S/MAR-DNA. Binding follows a cooperative mode, which is also typical for scaffold-S/MAR interactions (Kay and Bode 1994): each individual domain interacts only weakly with a DNA element, i.e. an UE according to Fig. 1. Only the simultaneous binding of SAF boxes to multiple UEs confers a strong and at the same time specific interaction in accord with the "mass-binding mode." This model explains the well-known phenomenon that there are hardly any naturally occurring S/MARs below a critical length of 250 bp. The failure of ssDNA to compete for the interaction of S/MARs with SAF boxes shows that SAF-box proteins alone cannot explain all criteria of scaffold-S/MAR interactions. However, a superimposition of SAF- and lamin-binding characteristics could well account for it.

Fackelmayer and colleagues (2004) have reported results from a defined model system to elucidate the role of scaffolds in DNA replication. They used extracts from *Xenopus laevis* eggs that contain all necessary components to assemble replication-competent nuclei, but do not support interfering reactions such as transcription or RNA maturation. They show, for the first time, that SAF-A provides an architectural framework on which active replication factories are assembled. Even when DNA is removed, SAF-A continues to form a nuclear reticulum. A dominant-negative approach indicates that the same is true in cultured cells. Consistent with its proposed role as a structural component, SAF-A/GFP fusions subjected to "fluorescence recovery after photobleaching" (FRAP) studies showed the protein to be rather immobile. Taken together, the data point to a structuring role of a SAF-A scaffold for DNA replication (congress report by Jackson 2005 and in preparation).

Meanwhile, SAF-A has been implicated in several more functions. Its association with histoneacetyltransferases (Martens et al. 2002) may be taken as an indication that S/MAR effects on histone hyperacetylation (Schlake et al.1994) are actually mediated by SAF-A implementing the protein in transcriptional potentiation (Sect. 3). Its involvement in the maintenance of nonviral episomes (Jencke et al. 2001) emphasizes its function in DNA replication.

3.2.1 SAF-A and Nuclear Hormone Receptor Functions

Nuclear hormone receptors are paradigms of regulated transcription factor systems that integrate signal transduction into nuclear architecture. The family includes receptors for steroid and thyroid hormones, for vitamin A and vitamin D. These receptors have become important pharmacological targets in a wide variety of clinical disorders, such as fertility issues and autoimmune diseases, which are now amenable to treatment with agonists or antagonists. It is anticipated that blocking the scaffold association of hormone receptors should have comparable effects to such an approach, but on a different level of regulation. Synergistic effects may arise when these treatments are used in combination (review: Fackelmayer 2004).

In the absence of a ligand most members of this receptor class localize to the cytoplasm. After hormone binding, they are translocated to the nucleus where they establish a punctuate pattern that is resistant to enzymatic removal of chromatin and thereby suggestive for nuclear matrix binding. It has been hypothesized that matrix-acceptor protein interactions occur in a cell-type-specific fashion. Identifying the acceptor proteins may therefore guide the development of specialized agents to modulate hormone-dependent gene expression patterns.

For the glucocorticoid receptor (GR), a minimal NMTS has been identified. If it was applied to screen for relevant acceptor proteins, SAF-A was recovered. Subsequent assays suggest that SAF-A might serve as the docking site for ligand-bound GR on the matrix. Comparable results are now available, demonstrating the specific interaction of a steroid hormone receptor with SAF-B, the second-most ubiquitous member of the SAF-box family in mammalia. When overexpressed, SAF-B exerts growth inhibition in breast tumor cells. In this context, it is noted that 20% of all breast tumors lack detectable levels of SAF-B, owing to aberrations at its gene locus (Fackelmayer 2004).

3.3 Nuclear Actin

Actin, actin-related proteins and numerous actin-binding proteins (including a nuclear-specific isoform of myosin I) are now known to be present in the nucleus, but their functions are emerging only slowly (Pederson and Aebi 2005). While there is no evidence yet for long actin filaments (F-actin), nuclear actin can form a multitude of dimers, short protofilaments and tubular, flat or branched oligomers. These nuclear actin polymers adopt a unique conformation that is recognized by specific antibodies. Since actins can also bind to two regions in the lamin A/C tail, they may be considered architectural partners of lamin filaments.

The presence of actin and nuclear myosin I (NMI) in the nucleus suggests a role for these motor proteins in nuclear functions. Although a direct participation in the nuclear matrix has remained uncertain, there have been hints for their interaction with nuclear RNAs and with proteins from hnRNP complexes (Percipalle et al. 2002), which, according to Kukalev et al. (2005), are essential for productive pol II transcription. Other studies demonstrate that antibodies against β-actin inhibit pol II transcription in a mammalian system for which actin was detected as a component of pre-initiation complexes and where it played a role in initiation (Hofmann et al. 2004). Since this inhibitory effect could be reproduced on naked DNA templates in vitro, the transcriptional role of actin does not seem to be restricted to chromatin remodelling complexes.

3.4 Nuclear Mitotic Apparatus Protein (NuMA)

NuMA, an abundant 240-kDa protein, binds to microtubules via its carboxyl terminal domain. During interphase, it is found in the nucleus, but during early mitosis it redistributes to the separating centrosomes. As a mitotic component it is essential

for the organization and stabilization of spindle poles up to the onset of anaphase. The cell cycle-dependent distribution and function of NuMA is regulated by phosphorylation (in mitosis) and dephosphorylation.

NuMA has been shown not only to bind S/MARs (Ludérus et al. 1994), but also to be part of nuclear matrix core filaments (Zeng et al. 1994). Its predicted structural features are a globular head, tail domains and a central two-stranded α-helical rod similar to members of IF family, suggesting that NuMA can form filamentous structures via coiled-coil interactions. In line with this expectation an extensive filamentous network of interconnected 5-nm fibers could be demonstrated upon transient over-expression (Harborth et al.1999). Antibodies against NuMA label portions of the nuclear matrix, but not on the 10-nm filaments. Since the actin-related nuclear proteins myosin and structural protein 4.1 bind to NuMA, this interaction may link actin-related and NuMA-related nuclear structures.

4 Regulatory Networks: Key Players Qualify by Diverse Interactions with S/MARs or Scaffolds

Our simplified view (Sect. 3.2) that constitutive S/MAR elements interact with constitutive proteins such as SAF-A or lamins does not exclude the modulation of these contacts by cell-type-specific factors. Besides, there are examples for proteins that gain or lose function by their integration into the matrix via an NMTS or by interaction with specific factors at the matrix. As an example, nuclear receptors have already been mentioned that gain activity by their association with SAF-A (Sect. 4.2.2). The proteins discussed below are involved in more diverse functions. They either interact with a plethora of other factors (PARP functions will be elaborated exemplarily since the scope of its actions is under extensive present investigation) or they are cell specific. A particularly well-studied example from the latter group is SATB1, the first member of a class of cell-type-specific fibre-forming components, which forms a functional link between the central topics of Sects. 4 and 5.

4.1 PARP

More than 40 years ago, Chambon et al. (1963) discovered that the addition of nicotinamide mononucleotide to rat liver nuclear extracts stimulates the synthesis of a polyadenylic acid, later identified as poly(ADP-ribose) (PAR). This discovery initiated research on the enzymes that regulate PAR metabolism, in particular poly (ADP-ribose) polymerase (PARP) and the de-modifying enzyme, poly(ADP- ribose) glycohydrolase (PARG). Since then 18 genes encoding members of the PARP family have been identified, among these the one encoding PARP-1, which accounts for 80% of this post-synthetic modification. PARP-1 forms homodimers, it catalyses the cleavage of NAD^+ into nicotinamide and ADP ribose, and it uses the latter to synthesize and attach nucleic acid-like polymers to Glu-residues in acceptor proteins, including

itself (automodification). The ribose-phosphate backbone of PAR has a higher negative charge even than DNA, enabling it to attract basic proteins with a specificity that is fine-tuned by its branched and helical structure. Nicotinamide, the smaller NAD^+ cleavage product, regulates PARP activity by feedback inhibition.

A still prevailing view is that the enzymatic activity of PARP-1 strictly depends on binding (via its double Zn-finger domain) to free DNA ends. This process triggers the allosteric activation of the enzyme leading to activities 10–500-fold above the so-called "basal state". A damage-induced activation alone, however, does not explain the multiple roles PARP-1 serves in the regulation of cellular functions under normal physiological conditions. Since the role of PARP-1 in DNA repair, apoptosis and necrosis has been reviewed extensively in the past (see Vidakovic et al. 2005b for a recent report from this group), the present article will emphasize PARP functions in transcription regulation. This does not exclude, however, phenomena at the intersection of both topics. As a whole, the chapter will serve to introduce and start to explain the beneficial therapeutic actions of PARP inhibitors in cancer and inflammation-related disease.

4.1.1 The Three as: Activation, Activities, and Actions

Activation: There are several levels at which the activity of PARP-1 can be regulated:

- By the classical pathway, i.e. by binding to single strand- and (even more strongly) to double-strand breaks
- By binding to certain DNA structures with base-unpairing and secondary-structure-forming potential
- By binding to DNA crossovers
- By activating factors that cause allosteric changes and/or prevent an automodification-dependent inactivation of the enzyme (Sect. 5.1.3)
- Other protein partners such as the lamins are storage sites for the inactive form of PARP-1 (Vidakovíc et al. 2004, 2005a,b)
    - PARP-binding sites in its own promoter (Soldatenkov 2002 and insert to Fig. 2), by interacting with lamins, may displace the PARP gene to nuclear periphery (lamina) and prevent its induction.

Recent evidence in fact emphasizes that, besides binding to DNA strand breaks, PARP-1 can also bind, in a cooperative manner, to the following DNA secondary structures: cruciforms, curved or supercoiled forms, crossovers and (either alone or as a complex with Ku antigen) to base-unpaired regions as found in S/MARs (Lonskaya et al. 2006). Some of these structures turned out to be even better activators than damaged DNA. The capacity of PARP-1 to bind to two DNA helices simultaneously (Rolli et al. 2000) indicates that the enzyme might be able to bind to the dyad axis where DNA enters and exits the nucleosome, providing an additional option by which its enzymatic activity can be regulated.

PARP-1 enzymatic activity is not only stimulated by the association with various DNA structures, but also by protein-binding partners. A well-investigated example

is its interaction with the transcription factor YY1, which can stimulate the enzyme as much as ten-fold (Griesenbeck et al. 1999). YY1 is accommodated by PARP-1 at its BRCT (breast cancer susceptibility protein C-terminal) domain. Interestingly, this motif overlaps its automodification domain such that part of the effect may be due to an impaired self-modification that would otherwise lead to PARP-1 inactivation. Actions of this kind might account for local heteromodification processes induced by the recruitment of PARP to particular genes.

Activities: PARP-1's catalytic domain supports several reaction types resulting either in linear or branched PAR polymers that can comprise as many as 200 units (D'Amours et al. 1999). In the absence of DNA damage, the length of the polymer is considerably shorter, ranging from single residues to oligo(ADP-ribose) units. Polymer size and complexity are determined by the relative contribution of the following activities:

- Initiation, i.e. the attachment of ADP-ribose to an acceptor protein
- Elongation
- Branching. The average branching frequency of the polymer is approximately one branch per linear repeat of 20–50 units of ADP-ribose (Alvarez-Gonzalez et al. 1999)

These activities may be regulated independently, at least to a certain extent: while the concentration of activating DNA motifs has been shown to affect the frequency of initiation, the polymer size is determined by the concentration of NAD^+ (Alvarez-Gonzalez et al. 1999). Therefore, it is conceivable that PARP-1 actions cannot only be adapted to the physiological state of the cell (as reflected by energy status and NAD^+ concentration), but also can be fine-tuned by allosteric regulators.

Actions: In line with some other proteins in this chapter (YY1, SATB1), PARP acts in a context-dependent manner (review: Kraus and Lis 2003), which means that the context determines whether it exerts activating or repressing effects:

- Each of the activating processes mentioned above has the potential to result in an opening of the respective chromatin domain, for instance by a local PAR modification of histones and non-histone proteins. Such a process facilitates transcription by RNA polymerases
- As an enhancer/promoter binding factor, PARP can support, *by association*, activators like YY1, p53, AP1, AP2, B-myb, TEF-1/Max, Sp1, Oct-1 and STATs
    - On the other hand, direct interaction with factors such as NFκ-B may inhibit the interaction with an DNA element (Chang and Alvarez-Gonzalez 2001)
- At elevated concentrations of NAD^+, PARP-1 can alternatively inactivate transcription factors such as YY1, p53, fos, SP1, CREB and TBP *by poly (ADP-ribosyl)ation*, which prevents their binding to the cognate sequences. These processes prevent initiation by Pol II, but they do not interfere with ongoing transcription
- Most of the activity-dependent effects are terminated by the PARP-1-specific automodification, which releases the enzyme from DNA or most of its interacting partners (for instance, NFκ-B, which, left alone, regains the capacity to associate with its cognate element)

PARP-1 can also serve structural roles in chromatin since it has a number of properties that are similar (but not identical) to H1, and it competes with H1 for binding to nucleosomes. In detail, PARP-1:

- Protects DNA in the linker region at the exit points of DNA from the nucleosome by binding via its double Zn finger (consistent with reports that PARP-1 can interact simultaneously with two DNA helices)
- Saturates nucleosome binding at a 1:1 molar ratio
- Increases the nucleosomal repeat length when binding to nucleosomal arrays
- In vivo, PARP-1 incorporation occurs into transcriptionally silent chromatin domains that are clearly distinct from histone H1-repressed domains. In this situation, it not only plays a structural role, but it is poised for NAD^+-dependent activation, which in turn leads to various levels of automodification, facilitating chromatin de-condensation and transcription by pol II

During these actions, transcription factors can become inactivated by poly-ADP-ribosylation and released from the DNA, thereby preventing repeated cycles of transcription. Such a process may be accompanied by histone-oligo(ADP-ribosyl)ation, an activity by which PARP-1 modulates chromatin structure. Among the histones H1 and H2B are the main substrates: while modified H1 is found associated with DNAsel hypersensitive sites (HS), core histones are the preferred targets if they are tightly bound to S/MARs. Combinatorial effects have also been described: certain acetylated H4 subspecies become predominantly tri- and tetra-(ADPribosyl)ated (Faraone-Mennella 2005). As usual, the series of events is terminated by extensive PARP automodification triggering its own dissociation from DNA.

The participation of PARP-1 in DNA repair has recently been reviewed (Vidakovíc et al. 2005b) and is briefly summarized in the lower section. The preferred occurrence of DNA strand breaks (*asterix*) at an HS is indicated (review: Bode et al. 2000a)

4.1.2 Autoregulatory Circuits

Only recent studies have suggested that a background PARP-1 activity in normal cells is an integral part of gene regulation during development and in response to specific cellular signals. As mentioned above, PARP-1 can act as a transcriptional regulator that mediates both positive and negative effects in a context-dependent manner. The latter phenomenon includes the suppression of its own promoter (Soldatenkov et al. 2002), for which we could verify the presence of S/MAR elements in several locations that were initially deduced from the BURs in an SIDD diagram (marked in the insert to Fig. 2). We could also show that the strongest (most destabilized) of the UEs mediates PARP binding and is thereby likely to be the responsible element (Vidakovic, in preparation).

Based on these data, we assume that the PARP-1 gene can be switched on upon demand, mostly to replenish the existing cellular pools consisting of non-activated

enzyme. From nuclear fractionation, crosslinking, immunoprecipitation experiments and fluorescence microscopy, we have deduced a functional interaction between non-modified PARP-1 and lamin B (Vidaković et al. 2004, 2005a). These results show that, under physiological conditions, a major part of the enzyme resides at the lamina shell. Since for mammalians the nuclear periphery represents a transcriptionally repressive compartment, it would appear conceivable that gene inactivation follows the association of the PARP promoter with the lamina.

While association with the lamina shell reflects the inactive state of PARP-1, its activation causes a gradual release as expected (Vidaković et al. 2005a). In the framework of our model, therefore, lamins are the major storage site of PARP-1. Both proteins share a high affinity for S/MAR sequences, and they preferentially recognize DNA secondary structures rather than a specific consensus (see Sects. 4.1 and 5.1.1). Because lamins belong to the substrates of PARP-1, these processes might be tuned in a combinatorial way by different degrees of poly(ADP-ribosyl)ation. This suggests that an extensive auto- and heteromodification supports the dissociation of PARP-1 from the lamina, enabling its engagement in other interactions. Dwelling of PARP-1 protein near S/MARs appears as a strategic advantage since S/MAR-associated DNA structures are a common feature of S/MAR-associated breakpoints (Bode et al. 2000a).

There are two reports for functional links between the major sections (transcription and repair) in Fig. 2.

Treatment of nuclei with RNAse or exposure to transcription inhibitors releases a subpopulation of PARP-1, indicating a role in transcription. Vispé et al. (2000) provide evidence for such a previously unrecognized pathway: PARP-1 is found to reduce the rate of transcription elongation by pol II, suggesting regulation at the level of PARP-RNA complexes. In damaged cells, binding of PARP to DNA breaks activates the enzyme in the presence of NAD^+, which promotes extensive automodification and results in its dissociation from DNA. This release allows DNA repair to commence. Since auto-modified PARP is likewise prevented from binding to RNA, mRNA synthesis is up-regulated. After the completion of DNA repair, unmodified PARP-1 will be regenerated by PARG to resume association with RNA.

A second mechanistic connection emerged between components of the DNA repair and transcription machineries: the signal-dependent activation of transcription by nuclear receptors and other classes of DNA binding transcription factors requires DNA topo IIβ-dependent, site-specific dsDNA breaks triggering PARP-1 activity. These transient breaks are in turn a prerequisite for local changes of chromatin architecture (Ju et al. 2006).

4.1.3 PARP and YY1

The function of Yin Yang 1 (YY1) 1 in transcription is context specific and requires interactions with many cellular factors. As a result, YY1 elaborates intracellular networks that allow it to induce multiple functions in transcriptional initiation, activation and repression, ultimately leading to the regulation of normal cell growth

and survival. Even the links to PARP-1 are multifold: YY1 activates the enzymatic activity of PARP-1 in a negative feedback mode as the process is terminated by PARP automodification (Oei and Shi 2001). Moreover, YY1 binding sites are present in the distal region of human PARP-1 promoter, suggesting that this factor participates in PARP-1- gene expression. These examples already suggest multiple functional relationships between both proteins in response to various stimuli.

YY1 was introduced by Guo et al. (1995) as "nuclear matrix protein 1" (NMP-1), i.e. as a DNA-binding factor with sequence-specific recognition of a regulatory element next to a histone H4 gene. Findings of this type suggest that YY1 may mediate gene-matrix interactions and participate in the assembly of multimolecular complexes. Using deletion constructs, McNeil et al. (1998) have demonstrated the role of the C-terminal domain in accord with data by Bushmeyer et al. (1998), who localized a nuclear matrix targeting signal to the Zn-finger region, a domain already known to mediate binding to DNA and to associate with components of the histone-acetylation apparatus (both HAT and HDAC are components of the nuclear matrix). It appears likely that at least some of these interactions are mutually exclusive; therefore, it will be of interest to determine how the association of YY1 with its binding partners is regulated and what role the related factor YY2 (Klar and Bode 2005) plays in this circuitry.

The precise localization of YY1/YY2-binding sites at the flanks of a destabilized region (insert to Fig. 1) deserves particular attention since it indicates the evolutionary conservation of both a potent binding motif and the ability to undergo strand separation. Although YY1 is a factor that requires both DNA strands simultaneously for its binding, this situation may require a flexible DNA backbone, the more so as certain actions of YY1 are ascribed to its DNA bending potential and the direction of the respective bend.

4.1.4 PARP, wt-p53 and mut-p53

Numerous studies have indicated a critical role for PARP-1 and p53 in the maintenance of genome integrity. Both proteins promote base excision repair (BER) via physical interactions with the BER protein complex. While the possible actions are manifold, both the ability of p53 to degrade mis-paired intermediates and the anti-recombinogenic function of PARP-1 may be relevant. PARP-1 exerts this function by protecting strand breaks before the repair complex has correctly been assembled. p53 has a high affinity for auto-modified PARP-1, which in turn is involved in the activation of p53 protein in response to DNA damage and other stimuli. These and numerous additional observations demonstrate that PARP-1 is an essential cofactor in the activation cascade of p53-dependent target genes.

Deppert and co-workers were the first to trace p53 to the nuclear matrix, while Jiang et al. (2001) have demonstrated that this association increases following DNA damage. In an effort to understand p53 functions by their relation to nuclear structures, Okorokov et al. (2002) found that a potential nuclear matrix component for such an association is nuclear actin (Sect. 4.3), the more so as this interaction is strengthened

during DNA damage. The dynamic interaction of p53 with the nuclear matrix is therefore one key to understanding the p53-mediated cellular responses to DNA damage.

Being a tumor suppressor gene, the presence of p53 reduces the occurrence of tumors by promoting apoptosis in cancer cells. A common pathway is the linear one involving bax transactivation, bax translocation from the cytosol to membranes, cytochrome c release from mitochondria and caspase-9 activation, followed by the activation of caspase-3, -6 and -7. p53 is modified by PARP early during apoptosis, leading to its stabilization. Only at later stages is PARP itself cleaved by caspase 3, and PAR is removed from p53 concomitant with the onset of apoptosis. On the

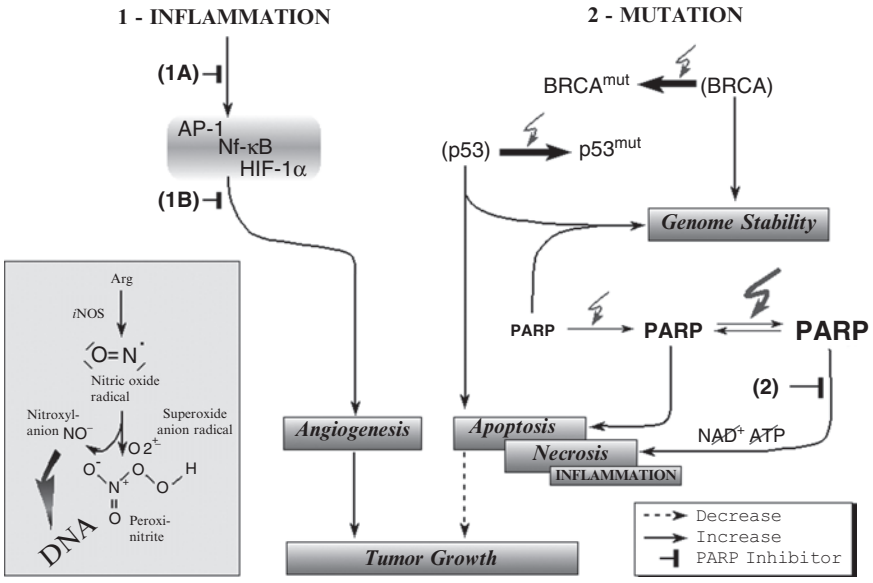

Fig. 3 PARP inhibitors counteract tumor growth and excessive inflammatory responses. Neutrophilic granulocytes mediate the activation of PARP-1 via the products of NO conversion (*insert*), which in turn supports the expression of pro-inflammatory peptides: activator protein 1 (*AP-1*) is a complex from c-jun and c-fos family members out of which the expression of c-jun becomes elevated during inflammatory lesions. NF-κB (p60 × p50) levels are rather constant, but the factor is kept in an inactive state due to association with I-κB. Inflammatory responses resolve the complex and cause NF-κB translocation to the nucleus. After their activation, both AP-1 and NF-κB induce their respective target genes (encoding inflammatory cytokines and matrix-degrading metalloproteinases). HIF-1 adapts leukocyte metabolism to low oxygen pressure as described in the text. PARP inhibitors prevent angiogenesis (and thereby tumor growth) by repressing both the pro-inflammatory (*1A*) and the induced inflammatory factors (*1B*). Regarding the effect of mutations, PARP-1 can, in part, substitute for the action of tumor suppressors (exemplified by p53) and cancer-susceptibility genes (exemplified by BRCA2) if these become inactivated. In this case, PARP-1 inhibitors serve to prevent the consequences from an overshooting activity (i.e. necrosis and subsequent inflammatory responses). If the severe depletion of NAD^+ and ATP pools can be relieved by PARP-1 inhibition, cells may enter the apoptotic pathway even in the absence of p53. If existing damage can be reversed, PARP may aid in approaching genome stability, at least to the extent that might be possible in the presence of p53-mutants

other hand, many apoptosis-related genes become transcriptionally regulated by p53. A full understanding of the mechanisms by which these and other factors induce apoptosis, and the reasons why cell death is bypassed in transformed cells, is of fundamental importance in cancer research and has great implications in the design of novel anticancer therapeutics (see Fig. 3).

While wt-p53 is well known for its tumour suppressor functions and for serving as a "guardian of the genome", mutation in the p53 gene is the most common event in cancer. This is the case for 80% of all colon tumors, 50% of lung tumors and 40% of breast tumors. A characteristic feature of its mutational spectrum is the frequency of missense point mutations at six hot spots within the region encoding the central DNA-binding domain of the protein. Most of these mutations not only lead to the inactivation of its tumor suppressor activity, but also confer oncogenic properties to its mutant forms (reviews: Deppert 1996; Kim and Deppert 2004). Actually, the transcriptional effects mediated by mut-p53 follow a mechanism totally different from p53 as they arise in the absence of specific responsive elements and affect an entirely different spectrum of genes that are either modulated in the positive or in the negative sense. It is intriguing that the search for *cis*-acting elements, which are targets of mut-, but not wt-p53, led to a variety of S/MARs and that the gain-of-function mutations in p53 could be correlated with the acquisition of a high S/MAR-binding potential ($K_d \sim 10^{-10}$ M). In Sect. 3 we have introduced S/MARs as regulatory DNA elements important for higher-order chromatin organization, long-range enhancer function and the propagation of chromatin modifications. This suggests that mut-p53/S/MAR interactions may serve to activate the expression of genes involved in cell proliferation and tumorigenesis, for instance, by forming a DNA loop that brings *cis*-acting regulatory elements into apposition with the respective promoters. Such a model might involve a p53-mediated sub-looping of chromatin loops for gene activation (Fig. 1) or the disruption of otherwise constitutive gene structures by interfering with the functions of domain borders.

4.1.5 PARP and BRCA1/2

In 1994, two human breast cancer susceptibility genes were identified: BRCA1 on chromosome 17 and BRCA2 on chromosome 13. Individuals with a mutation in either BRCA1 or BRCA2 are at risk of developing breast or ovarian cancer at some point in their lives. It was not clear what the function of these genes is until studies on a related protein in yeast revealed their normal role in the repair of radiation-induced breaks in double-stranded DNA. Now it is thought that mutations in BRCA1 or BRCA2 might disable this mechanism, leading to errors in DNA replication and ultimately to cancerous growth. Studies of *brca1*- and *brca2*-deficient cells in fact indicate that BRCA2 controls the intracellular transport and activity of RAD51, a protein necessary for double-strand break (DSB) repair by homologous recombination (HR). For BRCA1, the mode of action is less clear, but it might involve regulation of the MRE11 exonuclease that is required for creation ssDNA segments at the sites of DSBs.

Sub-cellular fractionation experiments showed a tight interaction with the nuclear matrix for most of BRCA1/2, and this localization was maintained following the treatment with DNA damaging agents that activate homology-mediated DSB repair pathways. Therefore, BRCA1 and -2 add to the list of examples where proteins act from positions that are anchored to the nuclear matrix. These data are consistent with models suggesting that components of specific repair complexes reside at the nuclear matrix to recruit damaged DNA (Huber and Chodosh 2005).

PARP1 activity becomes vital in BRCA2-deficient cells. As a result of their deficiency in HR, these cells are acutely sensitive to PARP inhibitors, presumably because collapsed replication forks are no longer repaired. This requirement can be exploited in order to specifically kill BRCA2-deficient tumours by PARP inhibitors. Such use of a DNA repair inhibitor for the selective killing of a tumour in the absence of an exogenous DNA-damaging agent represents a completely new concept in cancer treatment (Fig. 3).

4.1.6 PARP as a Therapeutic Target

The example of BRCA2 has introduced genes that counteract cancerous growth and for which PARP may play a complementary role. In other words, cells that lose one of these players have to rely on PARP for DNA repair and survival. If PARP is disabled, however, this will lead to the accumulation of DNA damage, causing the shift from repair to apoptosis (review: Vidakovíc et al. 2005b). This in turn may be the reason why PARP inhibition has proven beneficial in antitumour therapy. In a second scenario, damage might lead to the excessive over-activation of PARP. The associated depletion of NAD^+ and ATP would then result in necrosis and, as a consequence, to cell leakage and associated inflammatory responses. If PARP activity is reduced, cells might enter the apoptotic route instead, leading to the desired outcome.

Second-generation PARP inhibitors have now entered the field to support the chemotherapy and radiotherapy of human cancers (review: Virág and Szabo 2002). Their administration together with cytotoxic drugs that cause persistent single- and double-strand DNA has potentiated their activity. These principles are summarized in the right section of Fig. 3 (size of PARP lettering reflects PARP-1 activity).

4.1.6.1 PARP Inhibitors in Inflammation

Inflammation is the first response of the immune system to infection, irritation or other injury. Components of the immune system immediately infiltrate the affected site leading to an increased blood supply and vascular permeability. Neutrophils are at the forefront of cells to appear in the infected area where they trigger a local oxidant burst. This pulse is primarily directed against the invading agent, but it may give rise to pro-inflammatory factors, via DNA lesion/PARP activation, in nearby professional and nonprofessional immune cells. Peroxynitrite, a labile, toxic oxidant arising from the reaction of superoxide anion radical

and nitric oxide (NO), is considered to be the main trigger (Virág and Szabó 2002). These compounds as well as the nitroxyl anion and the hydroxyl radical induce DNA single strand breaks, leading to PARP-1 activity. In this context, it is noted that neutrophilic and eosinophilic granulocytes are the only known mammalian cells that do not contain PARP (Virág and Szábo 2002), probably since its presence would not be compatible with the high levels of local oxidant production they cause.

Different mechanisms have been proposed to explain the fact that an inhibition of PARP-1 improves the outcome of a variety of pathophysiological conditions associated with an irritated tissue and systemic inflammation:

- According to the "suicide hypothesis", the peroxynitrite-induced massive PARP-1 activation leads to a rapid depletion of NAD^+ and ATP. The same agent also induces mitochondrial free-radical generation, which amplifies these effects to trigger necrosis.
- While the extent of PARP-1 activation serves as a molecular switch between necrosis and apoptosis, the associated NAD^+ consumption acts as the metabolic link between DNA damage and cell death.
- Moderate PARP-1 activities promote transcriptional activation in lymphocytes. PARP-1 inhibitors have proven useful in this context and are applied for the treatment of autoimmune disorders, for instance, of the central nervous system.

Inflammatory actions disrupt the vascular structure around the site of injury and thereby lead to decreased oxygen pressure. The responding immune cells have to adapt to these conditions, and it has been found that leukocytes have the unique ability to cause a metabolic switch using HIF-1 (hypoxia-inducible transcription factor-1; Fig. 3). The PI3-kinase/Akt pathway and MAP kinase cascade, respectively, are involved in these pro-inflammatory responses. The adjustment of HIF-1 levels as well as the attenuation of NF-κB- and AP-1 actions by PARP inhibition has proven beneficial in this context, the more so as these measures restrict angiogenesis, which would otherwise support tumor growth (Martin-Oliva et al. 2006).

4.2 S/MAR-Dependent Interactions at the IgH Enhancer

Section 4.2 has shown that most of the ubiquitous proteins at the scaffold comply with BUR-binding characteristics (review: Galande 2002). While these proteins differ widely regarding their DNA-binding domains, it is noteworthy that most of them comprise a structural motif that might confer high affinity towards BURs. Recently, the SAF box could be localized in several DNA-binding proteins and can now be considered as one of the prototype domains. Another motif, the 11-residue "AT hook", was first detected in the HMG-I(Y) protein and associates with multiple A/T tracts that are separated by 6–8 bp, resembling the architecture of BURs. HMG1/2 and related proteins with multiple HMG boxes recognize irregular DNA structures in a sequence-nonspecific manner. One preferred substrate is the cruciform structure that arises by intrastrand

pairing at inverted repeats that are common in S/MARs (Mielke et al. 1996). As base unpairing is a prerequisite for cruciform formation, the latter process directly depends on SIDD properties. While the DNA binding features of PARP-1 and YY1 are governed by Zn-finger domains, the PARP-1/Ku70/86 complex recognizes S/MARs, probably as a consequence of its ss-DNA recognition potential (Galande and Kohwi-Shigematsu 1999). Our own laboratory has detected that YY1 consensus sequences with regulatory potential are consistently found adjacent to UEs, probably in order to utilize the factor's bending potential (see Sect. 5.1.3 and Klar and Bode 2005).

The following section will extend these aspects to cell-specific BUR binders in lymphocytes and discuss the regulatory networks of which they are part. Here, the immunoglobulin heavy-chain (IgH) locus has become a paradigm. Within the locus, transcription is controlled by promoters, located 5' of the individual variable (VH) gene segments, and by a composite downstream enhancer (Eμ). The Eμ region can be subdivided into an enhancer core (220 bp) and two 310–350-bp flanking S/MARs that were first defined by matrix-binding assays in vitro. According to our convention, these S/MARs are classified as being "facultative elements" (Fig. 1) as the associating factors, in striking contrast to the ubiquitous core-enhancer binding counterparts, are cell-type restricted. They have, therefore, the potential to cause dynamic changes of nuclear structures in accord with their suggested function.

4.2.1 SATB1

A factor binding to the 3′ Eμ-associated S/MAR in T-cells, special AT-rich DNA binding protein 1 (SATB1), became the founding father of all BUR binders when it was identified and cloned by virtue of its ability to bind to the core-unpairing element (CUE) located within the 3' S/MAR. SATB1 does not associate with mutated CUEs that lack the unwinding property (Bode et al. 1992). Since it neither binds to nor is competed off by ssDNA, the CUE must be recognized indirectly through an altered sugar phosphate backbone. Thereby it differs from other S/MAR-binding proteins such as mutant p53, which trigger a strand separation. We have anticipated, therefore, that its association would inhibit transcription, and our early data were in accord with this (somewhat simplistic) expectation (Kohwi-Shigematsu et al. 1997).

SATB1 comprises an unusual combination of a S/MAR-binding domain and a homeodomain, both of which are necessary for the recognition of the CUE. In addition, a dimerization motif is needed for BUR binding (Cai et al. 2003). This motif is homologous to PDZ domains, modular protein-binding structures with at least three distinct types of binding:

- Association with specific recognition sequences at the carboxy-termini of proteins
- Association with other PDZ domains to form heterodimers
- Homodimerization, which for SATB1 is one prerequisite for the recognition of BURs

The fact that a BUR-binding protein contains a putative PDZ domain has important biological implications as it is to be expected that SATB1 functions can be strengthened

or modulated by recruiting other PDZ-containing proteins to the S/MARs. This is the likely mechanism by which SATB1 builds up dynamic, cage-like structures within the nucleus (see below).

SATB1 was among the first cell-type-restricted S/MAR binders. It is expressed predominantly (but not exclusively: see Wen et al. 2005) in thymocytes where it represents one of the few gene products that are induced early upon peripheral T cell activation. A biological function emerged from the phenotype of SATB1 knockout mice, where SATB1 was found essential for orchestrating the spatial and temporal expression of a large number of T cell- and stage-specific genes: in the absence of SATB1, T cell development was severely impaired, and immature CD3-/CD4-/CD8- triple negative thymocytes were largely reduced in number. At the same time, SATB1-deficient thymocytes and T-cells in lymph nodes became prone to apoptosis.

The comparison of SATB1 knockout and wild-type mice indicated a role for SATB1 in the dysregulation of about 2% of the genes (Alvarez et al. 2000), also evidenced by the fact that the respective S/MARs were found to be detached from the nuclear matrix in vivo. These observations support the hypothesis that, in their normal context, specific genes are actively anchored to the nuclear matrix and that this association enables an appropriate regulation. Together these data show that there are factors acting as BUR-dependent regulators of cell function. Although the field is still in its infancy, other proteins that are readily detected on Southwestern blots or that are retained on BUR affinity columns may serve related functions at the bases of chromatin loop domains (Galande 2002).

How do SATB1 functions relate to details of the nuclear architecture? Cai et al. (2003) have demonstrated a then unknown nuclear distribution, i.e. a “cage-like” SATB1-containing structure circumscribing heterochromatic areas. They showed that this cage shares properties with a nuclear matrix as it resists the conventional extraction steps. The localization of the SATB1 network outside heterochromatic regions agrees with a model in which the attachment points (BURs) in the network provide landing platforms for chromatin-remodeling complexes (CHRAC, NURD), which constantly rearrange nucleosomes to support both positive and negative transcriptional actions. These pilot findings have widened our view into how a single protein can link the expression of hundreds of genes to nuclear organization.

4.2.2 SATB2

Dobreva et al. (2003) characterized a novel cell type-specific S/MAR-binding protein, SATB2, which is abundantly expressed in pre-B- and B-cells where it serves certain SATB1-like functions. SATB2 differs from its closely related thymocyte-specific relative by sumoylation-dependent modifications. Sumoylation is a recently detected post-translational modification system, biochemically analogous to, but functionally distinct from, ubiquitinylation as it involves the covalent attachment of a SUMO (small ubiquitin-related modifier) sequence to substrate proteins. Mutation of two internal conjugation sites (lysines) clearly enhances its activation potential

in parallel to the association with endogenous S/MARs in vivo, whereas N-terminal fusions with SUMO decrease SATB2-mediated gene activation. Since sumoylation is involved in targeting SATB2 to the nuclear periphery, this may cause modulation of SATB2 activities.

4.2.3 BRIGHT and NF-μNR

BRIGHT (B cell regulator of IgH transcription) is yet another factor transactivating the intronic IgH enhancer by binding to the S/MARs as a tetramer (Kaplan et al. 2001). BRIGHT contains regions homologous to the Drosophila SWI complex, suggesting that it might be involved in chromatin remodelling. A number of experiments suggest that the cell-type specificity of the Eμ enhancer is governed by negative regulatory mechanisms that are dominant to this and other B-cell specific transcriptional activators and that these actions can be ascribed to interference with nuclear matrix attachment. A responsible negative regulatory factor, first called NF-μNR and later found related to Cux/CDP, binds to multiple sites flanking IgH enhancer. Interestingly, the expression of NF-μNR displays a unique developmental pattern, as it is present in most cell lines outside of the B-cell lineage (T cells, macrophages and fibroblasts), but also in B-cells early in development. In contrast, it is absent from more mature cells that express high levels of IgH chains (Wang et al.1999).

5 Outlook and Perspectives

The term "epigenetics" was introduced in the 1940s by the British embryologist and geneticist Conrad Waddington as *the interactions of genes with their environment, which bring the phenotype into being*. Nowadays, the term refers to multiple modifications that influence gene activity without altering the DNA sequence. In the past, research has focused on transcription factors and signal transduction pathways associated with turning genes on and off. Only in the last decade scientists have touched the next layer in the flow of information, the more so as there was awareness of an "epigenetic code" that is central to processes such as development, aging, cancer, mental health and infertility. Since epigenetic changes are much easier to reverse than mutations, drugs that remove or add the chemical tags are at the forefront of cancer therapy.

The first epigenetic layer to be implicated in gene expression was DNA methylation. Drugs inhibiting DNA methyltransferases, such as Vizada (5-azacytidine) and the related Decitabine, are now established agents in the treatment of cancer, as they have the potential to re-activate specific tumour suppressor genes. Probably even closer to the steering centre are the elaborate principles of the "histone code", which determines how covalent histone modifications such as acetylation, methylation and PAR-addition modify chromatin accessibility to enable or tune gene expression (Hake 2004; Henikoff 2005). This code has become indispensable for

cancer specialists who utilize its principles to develop diagnostic tools and drugs. Virtually every major pharmaceutical company has meanwhile set up a program on histone decacetylases (HDACs) and their inhibitors that mediate chromatin opening by histone (hyper-)acetylation. The acquisition of Acton Pharma by Merck in 2004 provided access to SAHA (suberoylanilide hydroxamic acid), a second-generation drug of this class, and is only one recent example for such a commitment.

From a chemical viewpoint, all these histone modifications change the molecular crowding, electrostatic environment and other push-and-pull actions that are central to the "tensegrity concept" (Maniotis et al. 1997) and the "unified matrix hypothesis" (Scherrer 1989). Awareness is increasing that, ultimately, how chromatin works will turn out to be a scaffolding problem. The fact that histone modifications are mechanistically linked to DNA methylation and nuclear organization will inevitably lead to the merger of fields that, for a long time, existed side by side. Molecular mechanisms controlling gene expression include mechanical and chemical signal transduction pathways. In the framework of this view, it is the "*tensegrity"* (tensional integrity) that couples together the mechanical functions of filamentous structures, providing a balance between tension and compression. It could, in fact, be shown that mechanical stretch imposed upon a cell induces numerous biological responses, including alterations in the cytoskeleton, activation of cell signalling pathways and upregulation of transcription factors. The "unified matrix hypothesis" explains these phenomena by integrating the three skeletal networks, i.e. the extracellular matrix (ECM), the cytoskeleton (CS) and the nuclear matrix (NM), into a global scaffolding system.

Regarding the nuclear scaffold/matrix, one of the seminal findings is its dynamic aspects: although a fixed proportion of the total nuclear matrix proteins (NMPs) is always present, a subset of components, among these rare transcription factors, show variations according to the type and differentiation or transformation status of the cell. Here the structure serves to concentrate these proteins via NMTS signals or related principles and to deliver them to the respective S/MAR-associated control elements. The relevance of knowing about the identity of these specific NMPs became obvious when Fey and Penman (1988) documented a striking cell type specificity based upon a modified procedure for isolating the nuclear matrix-intermediate filament (NM-IF) portion of the nucleus. Soon thereafter, a correlation with cancer became apparent, explaining the observation that very often the cancer cell nuclei are oddly shaped. Antibodies served to detect cancer-specific NMPs that escape into body fluids in a process that is accelerated by the action of cytostatica. Since the late 1980s, these processes have been adapted to cancer diagnosis, and a test for NMP22 could be introduced based on the level of this protein in the urine of patients with bladder cancer (Getzenberg 1994). Subsequently, dedicated proteomics technologies provided access to NMP markers for cervical, breast, prostate and colon cancer. NMP tests of this type obviate any risk of infection, and they are now on the market for one sixth the cost of the cystoscopy-based routines.

Returning to the unified-matrix hypothesis, tumour cells from MLL rat prostate cancer were investigated with respect to their NMP profile. When these cells

were implanted at different organ sites, they developed a novel distinct and organ-specific NMP composition (Replogee-Schwab et al. 1996). Such dependence on the composition of proteins on the nuclear matrix is attributed to a tensegrity-based signal transduction, which initiates in the extracellular matrix microenvironment and terminates within the nucleus. This review has tried to provide a glance into the multiple influences that modulate nuclear matrix structures and functions.

A related aspect, the development of "chromosome-based" vectors, evolved in parallel to these efforts and has been reviewed before (Lipps et al. 2003). This field is dedicated to using chromosomal elements, and S/MARs in particular, to design independent regulatory units, so-called "artificial chromatin domains". After their integration, two bordering elements with insulating capacity will shield such a unit from the generally negative influences at the integration site (Bode et al. 1995; Goetze et al. 2005). An even greater challenge arose when it became possible to reduce the complexity of such a domain to a single S/MAR element and an actively transcribed gene. In the context of a circular (and hence supercoiled) construct, such a vector functions as a self-propagating (replicating) episome in the absence of any viral element. The recent demonstration that the performance of the system could be largely improved by deleting prokaryotic vector parts is considered a breakthrough: being composed of only eukaryotic modules, the resulting "minicircles" successfully escape the defense mechanisms of the host cell and show an extended–maybe unlimited–replication potential in the absence of selection pressure (Nehlsen et al. 2006). Again, it is the S/MAR that provides the link to the nuclear matrix (Jenke et al. 2001). In this position S/MARs not only enable the use of the endogenous transcription factories, but they also counteract silencing. Early observations link these properties to the episome's interaction with the histone acetylation machinery (Jenke et al. 2001).

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