Journal of Cellular Biochemistry Supplement 35:36–45 (2000)

Linking Chromatin Architecture to Cellular Phenotype: BUR-Binding Proteins in Cancer

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Key words: PARP; SAF-A; Ku 70/86; HMG-I(Y); breast cancer; MARs

Upon malignant transformation of cells, there are significant changes in expression of genes including those involved in growth regulation. Recent evidence showing that a base unpairing region (BUR)-binding protein can have global effects on gene expression involving a large number of genes has brought forth the possibility that proteins regulating chromatin structure may play a role in tumorigenesis. BURs represent a specialized DNA context with an unusually high propensity for base unpairing under negative superhelical strain. They are the hallmark of matrix attachment regions (MARs) that are thought to tether genomic DNA onto the nuclear framework or nuclear matrix, thereby forming topologically independent loop domains. Several proteins of known biological significance have been identified from cancer cells as BUR-binding proteins, including poly (ADP-ribose) polymerase (PARP), the autoantigen Ku70/86, SAF-A, and HMG-I(Y). Interestingly, the expression of these proteins is significantly elevated as the cancer takes on a more aggressive phenotype. This review discusses the perspective that BURs and BUR-binding protein interactions at the base of chromatin loops may serve as a functional and architectural core to influence multiple distant genes.

Certain oncogenes, such as c-*erb*B-2/*neu* and *p53*, are known to be aberrantly expressed in a significant portion of breast cancer [Slamon et al., 1989; Callahan, 1992]. However, a large population of patients with a poor clinical prognosis do not necessarily have these genes overexpressed [Isola et al., 1992]. There must be other proteins that are commonly present in all breast cancer cells and are involved in the uncontrolled growth of these cells. Cancer cells, generally characterized by uncontrolled cell growth and the ability to invade surrounding tissues, may have distinct ways in which chromatin is organized in order to assure certain sets of genes to be expressed or repressed ectopically or at different levels compared to their normal counterpart. The expression pattern of nuclear matrix proteins, for example, is considerably different between malignant and normal breast epithelial cells [Khanuja et al., 1993].

Specialized genomic DNA elements called MARs (sometimes called scaffold attachment regions or SARs) exhibit a high binding affinity to isolated nuclear framework or matrix in vitro. Accumulated evidence strongly suggest that in interphase nuclei, eukaryotic chromosomes are segregated into topologically independent loop domains formed by periodic attachments of MARs to the nuclear matrix [reviewed in Nelson et al., 1986; Gasser and Laemmli, 1987]. The biological significance of certain MARs in vivo in long-range chromatin structure and tissue-specific gene expression has been documented. In particular, studies on MARs flanking the

Contract grant sponsor: NIH; Contract grant number(s): R01CA39681; Contract grant sponsor: University of California Breast Research Program; Contract grant number(s): CABCRP 6JB-0135; Contract grant sponsor: Susan G. Komen Breast Cancer Research Grant; Contract grant number(s): 9524; Contract grant sponsor: DOE; Contract grant number(s): DE-AC03-76SF00098; Contract grant sponsor: The United States Army Breast Cancer Research Project DAMD (to SG); Contract grant number(s): 17-98-1-8303.

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Received 1 November 2000; Accepted 2 November 2000

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immunoglobulin heavy chain (IgH) enhancer [Cockerill et al., 1987] showed that these sequences are essential for the B-lymphocyte-specific transcription of a rearranged μ gene [Forrester et al., 1994]. These MARs have also been shown to generate long-range chromatin accessibility to transcription factors. This phenomenon correlates with extended demethylation of the gene locus in a transcription independent manner [Jenuwein et al., 1997]. In addition, B cell-specific demethylation at the immunoglobulin kappa (Igκ) gene locus requires both the intronic kappa enhancer and the nearby MAR [Lichtenstein et al., 1994; Kirillov et al., 1996].

We have been testing the hypothesis that there is a set of proteins that bind to common critical elements within MARs to dictate the loop domain structure of chromatin, thereby affecting expression of a large number of genes. Our idea is that cancer cells may express these proteins at different levels compared to their normal counterparts by organizing their DNA differently from normal cells to maintain their aggressive phenotype. A hallmark of these proteins is their binding specificity to a specialized DNA context with exceptionally high unwinding propensity by base unpairing when subjected to negative superhelical strain [Kohwi-Shigematsu and Kohwi, 1990; Bode et al., 1992]. Such DNA is designated BURs. BURs are typically identified in MARs and represented by a unique DNA context containing a cluster of ATC sequence stretches in which one strand consists of mixed As, Ts, and Cs, excluding Gs. The reason that such specialized regions of genomic DNA deserve attention are that (1) BURs, when they are in a double-stranded form, are specific targets of cell-type restricted proteins such as SATB1 [Dickinson et al., 1992] and Bright [Herrscher et al., 1995], while the mutated version that lacks the ability to unwind are not, (2) BURs, as the in vivo binding targets of SATB1, are localized at the bases of chromatin loops inside cells. These sites are firmly attached to nuclear framework inside nuclei after extensive extractions of proteins and DNase 1 digestion [de Belle et al., 1998], (3) SATB1 actively tethers BURs onto the nuclear framework [Cai and Kohwi-Shigematsu, unpublished results], and (4) the attachment of some of these genomic sequences onto the nuclear matrix inside cells is cell type dependent indicating that nuclear matrix attachment is not necessarily a fixed event, but rather it is dynamic [de Belle et al., 1998].

From the studies on SATB1 knockout mice, the first knockout of any MAR-binding protein, SATB1 was found to be essential for orchestrating the spatial and temporal expression of a large number of T-cell specific and developmental stage-specific genes and others [Alvarez et al., 2000]. In the absence of SATB1, T-cell development is severely impaired. This study has suggested that other BUR-binding proteins may have similar activity capable of affecting multiple genes at the bases of chromatin loop domains. In fact, a series of proteins that have similar DNA-binding specificity as SATB1 can be detected by either BUR affinity-column chromatography and Southwestern assay using a radiolabeled BUR probe.

SEARCH FOR BUR-BINDING PROTEINS ASSOCIATED WITH BREAST CANCER

We searched for BUR-specific binding proteins in malignant breast carcinomas and found that PARP, the autoantigen Ku (Ku 70/86), high mobility group proteins I and Y (HMG-I(Y)), and scaffold attachment factor-A (SAF-A) have binding specificity to double-stranded BURs. These proteins are expressed at dramatically higher levels in all malignant breast carcinomas tested compared with non-malignant breast epithelial cells. Furthermore, their expression levels correlated with the more advanced cancers with increased metastatic potential.

114 kDa Proteins (PARP and SAF-A)

More than 4 years ago Yanagisawa et al. [1996] reported identification of BUR-binding activity of a 114 kDa protein (p114), this activity was detected only in human breast carcinomas and not in normal and benign breast lesion tissues. The BUR-binding activity of the p114 kDa protein displayed an inverse correlation with the degree of differentiation of the carcinomas. Thus, a robust BUR specific-binding activity of PARP was detected in poorly differentiated rather than well-differentiated carcinomas (Fig. 1A and B). The p114 kDa protein was subsequently purified to near homogeneity from a breast carcinoma cell line SK-BR-3 using double-stranded BUR affinity chromatography. This protein was subsequently identified as PARP. Similar to SATB1, purified PARP

A

No.	TNM Staging		Lymph Node Status	Histologic Grading	p114 Activity
1	$T_2N_2M_1$	III_B	7/33	III	52.9
2	$T_4N_2M_1$	IV	n.a.	III	43.8
3	$T_2N_2M_0$	III_A	10/38	III	29.3
4	$T_2N_2M_0$	III_B	4/28	III	23.5
5	$T_4N_2M_1$	IV	34/67	III	23.4
6	$T_2N_1M_0$	II_B	3/23	III	22.4
7	$T_2N_1M_1$	IV	1/15	III	21.4
8	$T_2N_2M_0$	III_A	18/51	III	17.0
9	$T_4N_2M_1$	IV	12/37	III	16.0
10	$T_2N_1M_0$	II_B	3/21	III	15.5
11	$T_2N_0M_0$	II_A	0/13	III	14.7
12	$T_1N_2M_0$	III_A	3/34	III	14.6
13	$T_2N_1M_0$	II_B	3/40	III	13.3
14	$T_2N_1M_0$	II_A	2/29	III	13.3
15	$T_4N_1M_0$	III_A	12/15	(a)	10.7
16	$T_2N_0M_0$	II_A	0/18	III	10.3
17	$T_4N_2M_0$	III_B	24/32	II	24.0
18	$T_1N_1M_1$	IV	1/15	II	17.6
19	$T_2N_0M_0$	II_A	0/24	II	16.2
20	$T_2N_1M_0$	II_B	2/21	II	15.9
21	$T_2N_2M_0$	III_A	5/15	II	15.0
22	$T_4N_3M_1$	IV	0/31	II	13.0
23	$T_2N_0M_0$	II_A	0/32	II	12.4
24	$T_2N_0M_0$	II_A	0/39	II	12.3
25	$T_1N_1M_0$	II_A	1/22	II	11.8
26	$T_1N_0M_0$	I	0/26	II	10.2
27	$T_1N_2M_0$	III_A	2/29	II	8.4
28	$T_2N_0M_0$	II_A	0/12	II	7.3
29	$T_1N_1M_0$	II_A	1/29	II	5.5
30	$T_2N_1M_0$	II_B	1/24	II	5.0
31	$T_1N_0M_0$	I	0/33	II	3.9
32	$T_2N_1M_0$	II_B	2/17	I	5.0
33	$T_2N_1M_0$	II_B	2/11	I	5.0
34	$T_1N_1M_0$	II_A	5/14	(b)	4.8
35	$T_1N_1M_0$	II_A	9/52	I	1.0
36	DCIS		0/38		6.5
37	DCIS/microinvasion		0/39		5.5
38	DCIS/microinvasion		1/8		5.2
39	DCIS/microinvasion		0/13		4.6
40	DCIS/microinvasion		0/29		3.5
41	DCIS/microinvasion		0/34		1.5
42	DCIS/microinvasion		0/15		1.0
43	DCIS		n.a.		1.0

(a) infiltrating lobular carcinoma
(b) mucinous carcinoma

B

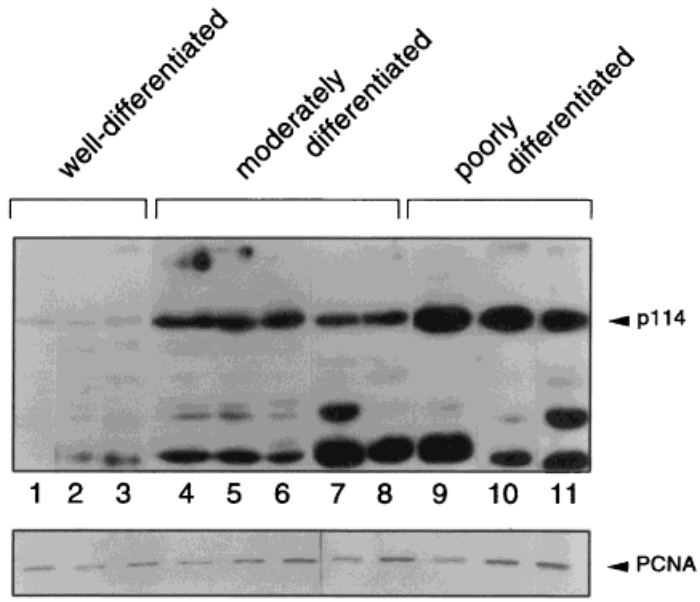

Fig. 1. Stronger p114 MAR-binding activity is associated with poorly differentiated breast carcinomas. **A**: Summary of data for 43 breast carcinoma specimens, including clinicopathological status. Relative MAR-binding activity was estimated by laser densitometric analysis of the p114 band on Southwestern blots. Histological grades are shown for infiltrating ductal carcinomas. Other types of carcinomas tested are: *a*, infiltrating lobular carcinoma; *b*, mucinus carcinoma. *No.* Tumor identification number. **B**: Southwestern blot analysis of breast carcinoma specimens. Forty micrograms of proteins extracted from breast carcinoma tissues were subjected to Southwestern blot analysis using radiolabeled WT $(25)_7$ probe. Well differentiated (**lanes 1–3**), moderately differentiated (**lanes 4–8**), and poorly differentiated (**lanes 9–11**) tumor samples. **Lanes 1–3**, tumor number 41–43; **lane** 4, tumor number 17; **lane 5**, tumor number 20; **lane 6**, tumor number 18; **lane 7**, tumor number 24; **lane 8**, tumor number 19; **lane 9**, tumor number 1; **lane 10**, tumor number 2; **lane 11**, tumor number 3. PCNA was used as a loading control.

exhibited strong affinity and specificity toward BURs but not to the mutated version of BURs that lack unwinding propensity [Galande and Kohwi-Shigematsu, 1999]. More recently, we identified one another protein with the same mobility as PARP that binds specifically to BURs [Galande, Lee, and Kohwi-Shigematsu, unpublished data]. This protein was identified as scaffold attachment factor A (SAF-A), which is hnRNP-U, a component of heterogeneous nuclear ribonucleoprotein complexes [Romig et al., 1992; Fackelmayer et al., 1994]. Thus, both PARP and SAF-A contributed toward the p114 BUR-binding activity detected by Southwestern analysis. Individual quantitation of PARP and SAF-A protein levels from breast carcinoma specimens by immunoblot analysis revealed that they were both proportional to the p114 MAR-binding activity [Galande and Kohwi-Shigematsu, unpublished observations].

Ku 70/86

The catalytic subunit of DNA-dependent protein kinase (DNA-PK_{CS}) and Ku 70/86, the DNA-binding heterodimeric subunits of DNA-PK, were specifically co-purified with PARP during BUR-affinity column chromatography. We found that PARP and Ku autoantigen form a molecular complex both in vivo and in vitro in the absence of DNA, and as a functional consequence, their affinity to BURs is synergistically enhanced. Either as individual proteins or as a protein complex, their specific recognition of BUR sequences was demonstrated by closed circular BUR-containing DNA templates that lack free ends. In contrast to the previous

notion that PARP and Ku autoantigen mainly bind to nicks and free ends of DNA, they were found to have much higher affinity and specificity to BURs.

HMG-I(Y)

Another pair of closely migrating BUR-binding proteins were detected on SDS-PAGE (appearing as a doublet around 20kDa) and their expression was well correlated with the aggressive phenotype of breast carcinoma cells with metastasizing ability [Liu et al., 1999]. The BUR-binding activity of these proteins is dramatically elevated in highly metastatic breast carcinoma cell lines, but is much lower in non-metastatic breast carcinoma cell lines. The 20 kDa proteins were identified as HMG-I(Y), a member of the high-mobility group (HMG) non-histone chromatin proteins. HMG-I and HMG-Y are splice variants of a single gene located on human chromosome 6p21 [Johnson et al., 1989], and are collectively referred to as HMG-I(Y). Purified recombinant GST-fused HMG-I and -Y proteins showed high affinity and specificity towards the BUR sequence [wild-type $(25)_7$] with an estimated dissociation constant (Kd) of 4×10^{-9}M (Fig. 2A, upper panel). In contrast, these proteins exhibited dramatically reduced binding affinity, by at least two orders of magnitude, to the non-BUR [mutated $(24)_8$] (Fig. 2A, bottom panel). It is known that HMG-I(Y) preferentially binds to the minor groove of A+T-rich DNA by recognition of substrate structure rather than nucleotide sequence [reviewed in Bustin and Reeves, 1996]. HMG-I(Y) was recently found to specifically recognize BURs rather than mutated BURs that are also A+T-rich [Liu et al., 1999]. Thus, HMG-I(Y) is similar to PARP, Ku, and SATB1 in terms of its DNA-binding specificity.

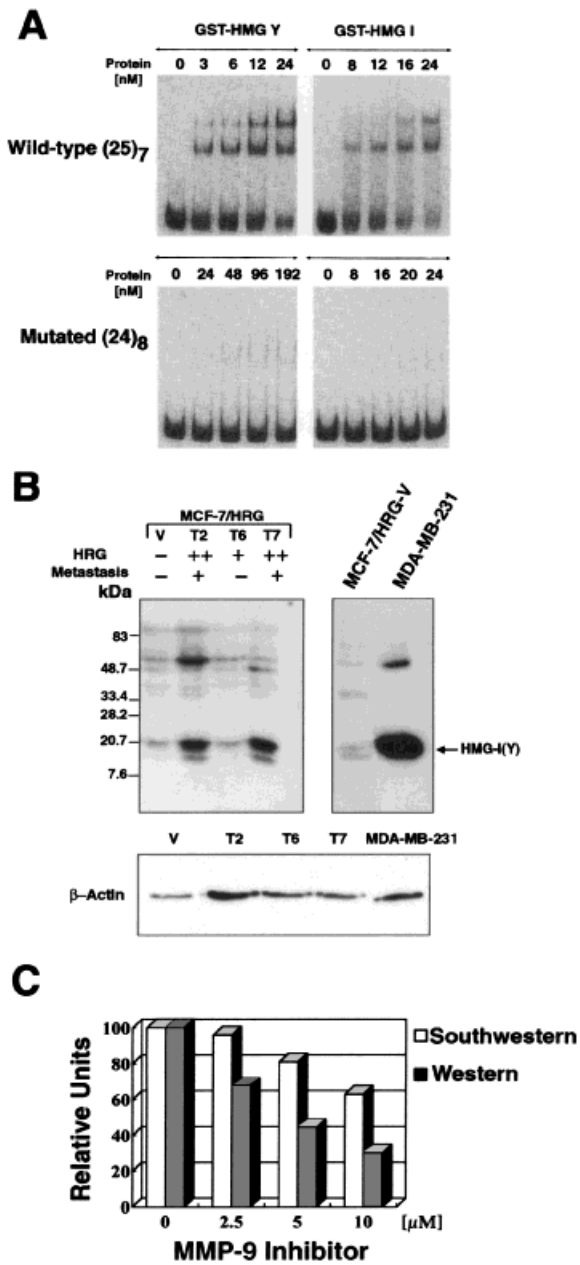

Fig. 2. HMG-I(Y) protein recognizes BURs regions of matrix attachment sequences and its increased expression is directly linked to metastatic breast cancer phenotype. **A**: Electrophoretic mobility shift assay was performed for GST-HMG-I and GST-HMG-Y with a radiolabeled wild-type $(25)_7$ probe (top panel), a mutated $(24)_8$ probe (bottom panel). The DNA probes were incubated with varying amounts of protein in 20 μl of binding reaction mixture. DNA–protein complexes were resolved on a 6% native polyacrylamide gel. Protein concentrations are indicated in nM. **B**: HMG-I(Y) expression is increased in MCF-7 cells upon HRG cDNA transfection. Twenty five micrograms of proteins extracted from MCF-7 and MDA-MB-231 cells were used for Southwestern analysis using a radiolabeled wild-type $(25)_7$ probe. MCF-7 cells transfected only with the pRC/CMV vector are indicated as 'V' in left upper panel and as 'MCF-7/HRG-V' in the right upper panel. Three MCF-7 clones transfected with HRG-β2 cDNA are indicated as T_2, T_6, and T_7. Identical amount of protein used in the Southwestern blot was subjected to immunoblot analysis using anti-β-actin antibody (lower panel). **C**: HMG-I(Y) expression is reduced in MDA-MB-231 cells upon MMP-9 inhibitor treatment. Twenty micrograms of proteins extracted from MDA-MB-231 cells with MMP-9 inhibitor treatment for 6 days at indicated concentration (0, 2.5, 5, and 10 μM) were used for Southwestern analysis using a radiolabeled wild-type $(25)_7$ probe and western blot analysis using anti-HMG-I(Y). Southwestern and western blots were quantitated by phosphorimager and laser densitometer analysis respectively. Signal intensities are expressed as relative units.

POTENTIAL BIOLOGICAL SIGNIFICANCE OF PARP AND KU ASSEMBLY ON BURS

Both PARP and DNA-PK are activated by DNA strand breaks and have been implicated in DNA repair, recombination, DNA replication, and transcription. For example, the biological roles of PARP and DNA-PK in DNA repair and V(D)J recombination are linked by their common property of DNA break-induced activation [reviewed by de Murcia and Menessier-de Murcia, 1994; Jackson, 1996]. Studies utilizing PARP and DNA-PK deficient cells have suggested that these two proteins functionally cooperate to mitigate genomic damage caused by double-stranded breaks [Morrison et al., 1997].

The DNA-binding domains of PARP and Ku70/86 differ significantly, however these two proteins remarkably bind to BURs as their common target. The protein complex formed on BURs might be much larger since PARP is known to physically interact with p53 [Vaziri et al., 1997], XRCC1 [Masson et al., 1998], DNA polymerase α-primase complex [Dantzer et al., 1998], while Ku 70 interacts with Mre 11 [Goedecke et al., 1999] and the DNA-PK_{CS} interacts with XRCC4 which in turn is shown to interact with ligase IV [Leber et al., 1998]. Some of these proteins, for example XRCC4 and ligase IV, are specifically involved in joining the double-strand breaks. Interestingly, the major breakpoint region (MBR) in the untranslated portion of the *BCL2* gene contains ATC sequence stretches that bind to SATB1 in vivo [Ramakrishnan et al., 2000], suggesting that BURs might also be sites for recombination attracting proteins that repair double-strand breaks. Along with SATB1, several other proteins formed complexes with this sequence. Most strikingly, PARP, DNA-PK_{CS}, and Ku 70/86 were also found to be the associating partners in this multiprotein complex at the MBR, presumably tethered to the matrix [Ramakrishnan et al., 2000]. The high affinity of Ku70/86 and PARP for BURs, and their synergistic binding to these regions may also aid in the recruitment of many of the above proteins with which they physically interact, culminating in the assembly of a multiprotein machine at BURs.

The formation of the protein complex on BURs containing PARP may be dynamically regulated by protein modification. Poly (ADP-ribosyl)ation may serve as a toggle switch determining the protein composition of the BUR-binding complex. ADP-ribosylation of DNA-PK by PARP in vitro stimulated its kinase activity, suggesting their functional interaction in response to DNA damage [Ruscetti et al., 1998]. We have shown that ADP-ribosylation of PARP resulted in the loss of PARP's BUR binding activity and abrogated the assembly of the PARP and Ku complex on BUR [Galande and Kohwi-Shigematsu, 1999]. The individual protein components of the multiprotein BUR-associated complex may shuttle between the bound and free state depending upon their covalent modification. Additionally, PARP is also involved in non-covalent association with other proteins through poly (ADP-ribose) (PAR) polymer. Recently, Pleschke et al. [2000] have identified a PAR-binding motif in several DNA damage checkpoint proteins such as p53, XRCC1, Ku 70, DNA PKcs, Ligase III, DNA polymerase ε, p21, XPA, MSH6, NF-κB, iNOS, CAD, and telomerase. The PAR-binding domain overlapped with functional domains in these proteins that are responsible for protein–protein interactions, DNA-binding, nuclear localization, nuclear export, and protein degradation. Thus, PARP may target-specific signal network proteins via poly (ADP-ribose) and regulate their function. Furthermore, poly (ADP-ribosyl)ation also suppresses RNA polymerase II-dependent transcription [Oei et al., 1998], suggesting that PARP may play a central role in facilitating recovery from DNA damage by silencing transcription and stimulating DNA repair.

PARP was also found to be a component of the mutiprotein DNA replication complex (MRC) including DNA polymerase α, DNA topoisomerase I, and PCNA which catalyzes replication of viral DNA in vitro [Simbulan-Rosenthal et al., 1998]. In addition, PARP appears to recruit PCNA and topoisomerase I into MRC. PARP also regulates expression of the transcription factor E2F-1, which positively regulates transcription of DNA polymerase α and PCNA genes [Simbulan-Rosenthal et al., 1998]. Therefore, high expression of PARP in aggressive breast carcinomas would support the high proliferation rate of carcinomas. However, upregulation of PARP in malignant cells is apparently not solely explained by the high proliferation activity of these cells because similarly proliferating non-malignant cells in

culture express dramatically lower levels of PARP [Galande and Kohwi-Shigematsu, unpublished observations].

BUR-BINDING HMG-I(Y) IS DYNAMICALLY REGULATED BY VARYING TYPES OF SIGNALING THAT AFFECT METASTATIC ABILITY

Most differentiated normal mammalian cells and adult tissues express extremely low levels of HMG-I(Y) mRNA and protein and elevated HMG-I(Y) gene expression is correlated with advanced cancers [reviewed in Wunderlich and Bottger, 1997]. This resembles the case of PARP where the BUR-binding activity of which is hardly detectable by Southwestern analysis of these cells [Yanagisawa et al., 1996]. Since growth factors and their receptors play an important role in cancer growth and maintenance, we examined whether HMG-I(Y) expression responds to signaling using testable models. These studies have shown that the expression of HMG-I(Y) is regulated dynamically in response to various types of signaling that affect metastatic ability, including heregulin (HRG) and extracellular matrix (ECM).

HRG is a growth factor originally isolated from hormone-independent and invasive breast cancer cells [reviewed in Lupu et al., 1996]. It activates one of the *erb*B-receptors, *erb*B-2, overexpression of which correlates with a poor prognosis, most notably in breast cancer [Lupu et al., 1996]. MCF-7 cells, which are non-tumorigenic in the absence of estrogen and non-metastatic in nude mice even in the presence of estrogen, can be converted into a more aggressive phenotype and rendered both tumorigenic and metastatic in vivo by transfecting them with an HRG expression construct [Tang et al., 1996]. Thus, this provides a breast cancer progression model. Conversely, transfecting highly metastatic MDA-MB-231 cells with an antisense-HRG construct reverses their highly invasive phenotype to non-invasive phenotype [Azzam et al., 1993]. The levels of HMG-I(Y) were determined in these two cell model systems. Among individually isolated HRG-transfected MCF-7 cell clones, a significant increase in the HMG-I(Y) protein levels was found to be strictly correlated with the acquired metastasizing capability of cells (Fig. 2B), and not the high proliferation rate of the cells as both metastasizing and non-metastasizing cell clones had a similar proliferation rate. Consistent with this observation, in the antisense HRG construct-transfected MDA-MB-231 cells, the HMG-I(Y) level decreased concomitantly with the loss of their metastatic phenotype due to the disruption of HRG expression. These data show that elevated HRG induces HMG-I(Y) expression and metastatic potential of breast cancer cells is associated with elevated HMG-I(Y) [Liu et al., 1999]. Changes in the level of HMG-I(Y) accompanied morphological alternations of cells. This is consistent with the notion that aberrant nuclear and cellular structures, which are hallmarks of malignant transformation, alter chromatin structure [reviewed in Holth et al., 1998 and Stein et al., 2000].

The effect of ECM-mediated signaling on HMG-I(Y) expression was also monitored. Proteases that degrade the ECM, including the serine proteases and matrix metalloproteinases (MMPs) gelatinase A (MMP-2) and B (MMP-9), have been implicated in cancer growth, invasion, and metastasis [Azzam et al., 1993; Zucker et al., 1993]. For example, inhibition of MMP-9 expression using a ribozyme has been shown to prevent metastasis in the rat sarcoma model system [Hua and Muschel, 1996]. We have shown that blocking MMP-9 activity in an aggressive breast cancer cell line, MDA-MB-231 cells, with an MMP-9 chemical inhibitor resulted in a loss of invasive phenotype in vitro and a decrease of the HMG-I(Y) protein levels (Fig. 2C) [Liu et al., 1999]. These data strongly argue in favor of a signaling link between metalloproteinase activity at the ECM and nuclear events such as the HMG-I(Y) expression, which may in turn cause widespread changes in gene regulation.

LINKING CHROMATIN STRUCTURE AND FUNCTION BY BUR-BINDING PROTEINS

In summary, there is a short list of proteins that have been shown to possess a high affinity and specificity to the BUR DNA context. They make a clear distinction between BURs and their mutated versions which have lost the unwinding propensity. These are PARP and Ku 70/86 heterodimer [Galande and Kohwi-Shigematsu, 1999], HMG-I(Y) [Liu et al., 1999], SAF-A [Galande Lee and Kohwi-Shigematsu, unpublished observations], nucleolin [Dickinson and Kohwi-Shigematsu, 1995], SATB1 [Dickinson

et al., 1992], and Bright [Herrscher et al., 1995]. The first four proteins have been shown to be associated with cancer. In addition to these four, it is noteworthy that mutant, but not wild-type p53 binds with high affinity to a variety of MARs [Müller et al., 1996]. Since at least some mutant p53 proteins identified from various types of cancer have not simply lost the wild-type p53 specific tumor suppressor function, but exhibit oncogenic functions on their own [reviewed in Roemer, 1999], the newly acquired MAR-binding activity of mutant p53 may be linked to its "gain-of-function" phenotype. Although mutated p53 does not show specific binding to double-stranded BURs compared to the mutated BURs, addition of mutated p53 to the mixture of double-stranded and single-stranded BUR oligonucleotides resulted in a dramatic reduction in the double-stranded form, suggesting BUR-specific strand separation [Will et al., 1998].

It is of great interest to understand the function of BUR-specific proteins in tumorigenesis. Some important hints have been provided from studies using SATB1 knockout mice. In contrast to wild-type cells containing SATB1, in SATB1-deficient cells, the in vivo binding target BURs near dysregulated genes remain detached from the nuclear matrix [Cai and Kohwi-Shigematsu, unpublished results]. Thus, SATB1 was found to actively anchor a specific group of BURs onto the nuclear matrix and presumably this leads to the proper regulation of a large number of genes. Some BUR-binding proteins listed above may have a BUR anchoring activity similar to SATB1 for forming a specific loop domain structure. Some of the above BUR-binding proteins may not have such an anchoring activity, but merely bind to BURs that are already attached to the nuclear matrix. Even in such a case, BUR-binding proteins may still facilitate recruitment of a larger protein complex at BURs. In support of this view, HMG-I(Y) is known to be an architectural transcription factor that assembles multiple proteins and is involved in both positive and negative regulation of many genes [Bustin and Reeves, 1996]. Recent work has shown that HMG-I(Y) regulates gene expression by changing the topology of DNA by promoting DNA loop formation establishing its link with chromatin structure [Bagga et al., 2000]. In this case, however, the loop formation was shown in vitro without the involvement of the nuclear matrix. HMG-I(Y) is hardly detected as a component of the nuclear matrix in contrast to SATB1, for example [de Belle et al., 1998; our unpublished results]. We speculate that HMG-I(Y) potentially plays a role in the subloop domain structure formation within a large loop domain formed by interaction of DNA with the nuclear matrix. SAF-A has been shown to exhibit "bundling" activity, which brings together multiple DNA sites forming large aggregates [Romig et al., 1992]. This would bring distant loop domains into close proximity. Any differences in the expression levels of BUR-binding proteins, whether they have nuclear matrix anchoring activity or bundling activity, could thus lead to changes in expression of multiple distant genes. Furthermore, BURs might be the sites at which large protein complex formation takes place as discussed below. A model that schematically shows how BUR and BUR-binding protein might link chromatin structure and function is shown (Fig. 3). It is significant to note that nuclear BUR-binding proteins that are capable of influencing a large number of genes

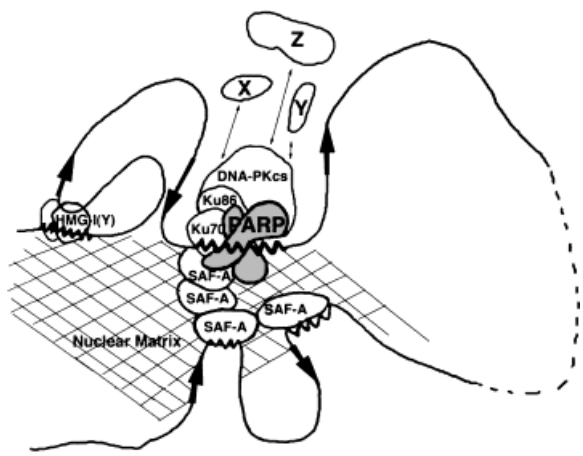

Fig. 3. Schematic model showing how BUR-binding proteins might interact with genomic DNA to affect function of multiple genes. Solid line indicates DNA, wavy line indicates BURs, and thick arrows indicate genes. Assembly of a BUR with PARP, Ku70/86, and DNA-PKcs would recruit many other proteins, X, Y, Z, as described in the text. HMG-I(Y) binding to another BUR may form a subloop within a topologically independent loop domain defined by attachment of BUR to nuclear matrix. SAF-A is a component of nuclear matrix and has a property to self-associate into filamentous polymers in the presence of DNA [Fackelmayer et al., 1994]. Therefore, SAF-A potentially brings together distant BURs. Transcription factors and proliferation factors that are presumably assembled onto BURs can thus affect multiple distant genes. Such protein assembly at BURs and loop formations are considered dynamic and probably regulated by various protein and DNA modifications.

can be regulated by various types of signaling that affect the invasiveness of cancer. In breast cancer and other types of cancer, a significant change in the levels of certain BUR-binding proteins reflecting the stages of malignancy may be essential for the global changes in gene expression required during progression of the disease.

FUTURE PERSPECTIVES

There are number of crucial questions that remain to be investigated. Do BUR-binding proteins, by interacting with BURs, promote long distance effects of enhancer or repressor by changing specific chromatin structure in vivo? If so, what are the specific changes in chromatin structure induced by these proteins and how are these structural changes promoted by BUR-binding proteins? Which genes are directly regulated by BUR-binding proteins in malignant cells? What are the roles of BUR-binding proteins in replication in addition to transcription?

Based on accumulated evidence, Peter Cook proposed the concept that replication and transcription factories are organized in interphase nuclei by being immobilized through attachment to the nuclear framework. The immobilized protein complexes reel in their templates and extrude newly made nucleic acids [Cook, 1999]. BUR sites may serve as a scaffold for the assembly of at least some of these multiprotein complexes or factories and such assembly may be mediated by BUR-binding proteins. For example, PARP is part of the multiprotein DNA replication complex or DNA synthesome [Simbulan-Rosenthal et al., 1998]. Similarly, SATB1, which is responsible for repressing a large number of genes, recruits multiple protein complexes onto BURs [Yasui and Kohwi-Shigematsu, unpublished data]. It is important to characterize each of the components of the protein complex formed at a given BUR and the effect of this assembly on the degree of chromatin folding, chromatin modifications such as methylation, acetylation, and ribosylation, to understand how such protein assembly affects distant gene expression. In the future, identification of other BUR-binding proteins, both cell type specific and ubiquitous as well as carcinoma-associated, would help obtaining a better outlook toward their potential function. The basic research outlined above is expected to shed light onto the mechanism of tumorigenesis at the chromatin structure level and help design ways to control cancer cells.

ACKNOWLEDGMENTS

We thank Drs. Dag Yasui and David Chen for helpful comments on the manuscript.

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