PROSPECT
Journal of Cellular Biochemistry 109:312–319 (2010)

Journal of Cellular Biochemistry

SAFB1's Multiple Functions in Biological Control—Lots Still to Be Done!

Jason P. Garee[1,2] and Steffi Oesterreich[1,3,4*]

[1]Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, Texas
[2]Graduate Program of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas
[3]Department of Medicine, Baylor College of Medicine, Houston, Texas
[4]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas

ABSTRACT

The examination of scaffold attachment factor B1 (SAFB1) and its multiple functions and tasks in cellular processes provides insight into its role in diseases, such as cancer. SAFB1 is a large multi-domain protein with well-described functions in transcriptional repression, and RNA splicing. It is ubiquitously expressed, and has been shown to be important in numerous cellular processes including cell growth, stress response, and apoptosis. SAFB1 is part of a protein family with at least two other family members, SAFB2 and the SAFB-like transcriptional modulator SLTM. The goal of this prospect article is to summarize known functions of SAFB1, and its roles in cellular processes, but also to speculate on less well described, novel attributes of SAFB1, such as a potential role in chromatin organization. This timely review shows aspects of SAFB1, which are proving to have a complexity far greater than was previously thought. J. Cell. Biochem. 109: 312–319, 2010.

KEY WORDS: SAFB1; FUNCTIONS; BIOLOGICAL CONTROL

Renz and Fackelmayer [1996] cloned the scaffold attachment factor B (SAFB) based on its ability to bind matrix/scaffold attachment regions (S/MARs). At the same time, we identified the same gene in a screen for proteins binding to the promoter of the small heat shock protein hsp27, and termed it HET, for hsp27 ERE TATA-binding protein [Oesterreich et al., 1997]. Weighardt et al. [1999] subsequently identified SAFB/HET in a yeast two-hybrid screen utilizing hnRNP as bait. They named the protein HAP (hnRNP A1 associated protein). HET, SAFB, and HAP are identical. With the identification of a second family member, SAFB2 (see discussion below), the Human Genome Organization (HUGO) Gene Nomenclature Committee approved the naming of the proteins as SAFB1 (the original SAFB/HET) and SAFB2. For the purpose of this prospect review this nomenclature will be followed with the term "SAFB" being used in those instances where there is no distinction between SAFB1 and SAFB2.

SAFB FAMILY MEMBERS

SAFB1 exists in a protein family; the second family member, SAFB2, shares 74% similarity with SAFB1 at the amino acid level, with the scaffold attachment factor-box (SAF-box) (also known as SAP domain for SAF-A/B; acinus; and PIAS domain) and RNA recognition motif (RRM) domains sharing higher similarities [as reviewed by Oesterreich, 2003] (Fig. 1). SAFB1 and SAFB2 are encoded by two separate genes separated by a 490 bp promoter oriented in a bidirectional divergent fashion mapping to chromosome 19p13.3 [DuPont et al., 1997; Townson et al., 2003].

More recently, a third family member SLTM [SAF (scaffold attachment factor)-like transcription modulator] has been identified and characterized [Chan et al., 2007]. SLTM shares 34% overall identity with SAFB1 and 36% with SAFB2 [Chan et al., 2007 and unpublished observations]. SLTM contains SAP and RRM domains which share higher similarities of 60% and 70% respectively with SAFB1. The presence of these shared domains suggests at least some common functions among SAFB1, SAFB2, and STLM; however, limited information is available on additive, synergistic, or potentially antagonist functions of the family members.

The SAFB family members are ubiquitously expressed; using RNAse protection analysis, we have previously shown that SAFB1 and SAFB2 are expressed in most tissues, with very high expression

Grant sponsor: NIH; Grant numbers: R01 CA097213, P01030195.
*Correspondence to: Steffi Oesterreich, Breast Center, Baylor College of Medicine, Houston, TX.
E-mail: steffio@bcm.edu
Received 14 October 2009; Accepted 15 October 2009 • DOI 10.1002/jcb.22420 • © 2009 Wiley-Liss, Inc.
Published online 11 December 2009 in Wiley InterScience (www.interscience.wiley.com).

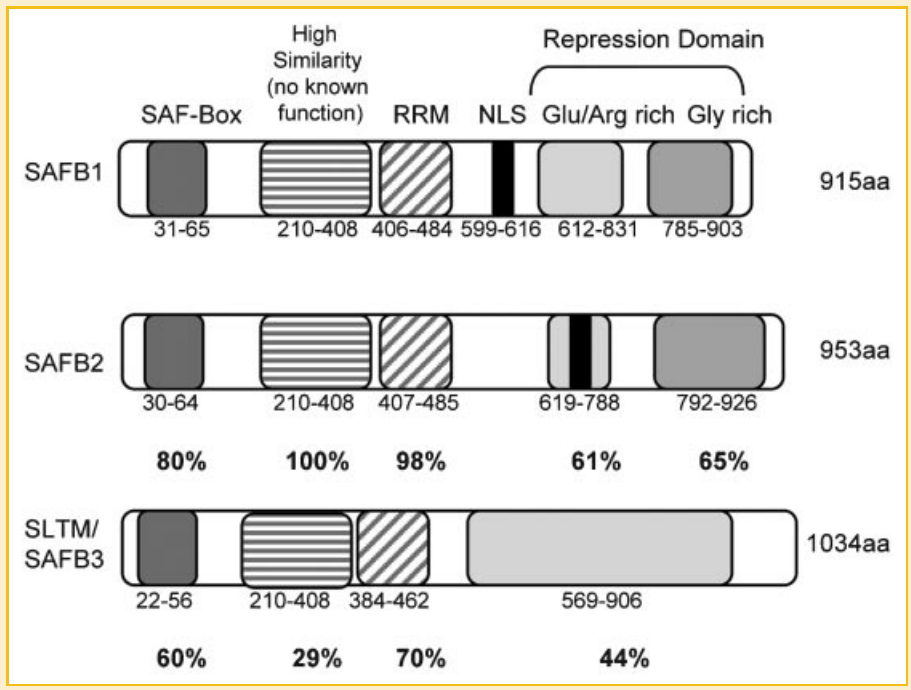

Fig. 1. Schematic of SAFB family members. Important known domains of the SAFB family (SAF–box, RRM, NLS) are indicated. Additionally, a region of high similarity with no known function is noted. Similarity of domains of SAFB2 and SLTM relative to SAFB1 are indicated. Amino acid positions of indicated domains are listed.

in brain [Townson et al., 2003]. Further in silico studies have indicated ubiquitous expression of all three SAFB family members (Fig. 2). Interestingly, these studies have indicated some unique expression patterns of the three family members. For example within the bone marrow SAFB1 is only expressed in cancerous conditions while SAFB2 is expressed only in normal condition and SLTM is expressed in both. This suggests some unique functions of each family member with respect to the others. Data about differential expression (either unique expression or lack of expression) identified through in silico approaches is summarized in Figure 2.

In general, SAFB2 and SLTM are less well characterized than SAFB1, and thus in this prospect review we will mostly focus on SAFB1.

SAFB1 AS A MULTI-FUNCTIONAL PROTEIN

SAFB1 is a large multi-domain protein with multiple functions including transcriptional regulation, RNA splicing, and a proposed role in chromatin organization. The N-terminus contains a SAF-box which is important for binding to AT-rich S/MARs. The SAF-box is often found in proteins involved in the regulation of higher order chromatin structure [Kipp et al., 2000]. The central region contains an RRM, for binding to RNA and/or single stranded DNA, and a nuclear localization signal (NLS). The C-terminus was originally identified to contain Glu/Arg- and Gly-rich regions, often involved in protein–protein interaction. Additional studies identified that the C-terminal region contains a potent and transferable transcriptional repression domain [see discussion below and Townson et al., 2004].

SAFB1 IN TRANSCRIPTIONAL REPRESSION

We originally identified SAFB1 based on its ability to bind to, and repress the activity of the hsp27 promoter. The hsp27 promoter region contains an estrogen response element (ERE) which suggested a potential link between SAFB1 and estrogen receptor alpha (ERα). Indeed, subsequent studies indicated that SAFB1 can bind to ERα and repress it transcriptional activity [Oesterreich et al., 2000a,b; Townson et al., 2004]. This co-repressor action is mediated via SAFB1's intrinsic C-terminal repression domain. These findings moved SAFB1 into a steadily growing family of coregulator proteins that can either positively (coactivators) or negatively (corepressors) modulate transcriptional activity [as reviewed by Lonard and O'Malley, 2007]. Given the critical role of coregulators in controlling transcription, it is not surprising that many have been shown to be important in a variety of human diseases [Lonard et al., 2007].

SAFB1's role as a corepressor of nuclear receptors is not limited to ERα. Studies performed by the Gelman group have shown that SAFB1 can interact with and repress transcriptional activity of various other nuclear receptors including PPARγ, FXRα, RORα1, PPARα, PPARβ, VDR, SF1, and LRH-1 [Debril et al., 2005]. These data clearly suggest that SAFB1 affects multiple targets and therefore loss of SAFB1 activity may lead to aberrant signaling of multiple pathways.

In addition, there is recent evidence that SAFB1 can regulate transcription independent of its interaction with nuclear receptors. For example, Lin et al. [2008] and Omura et al. [2009] showed that

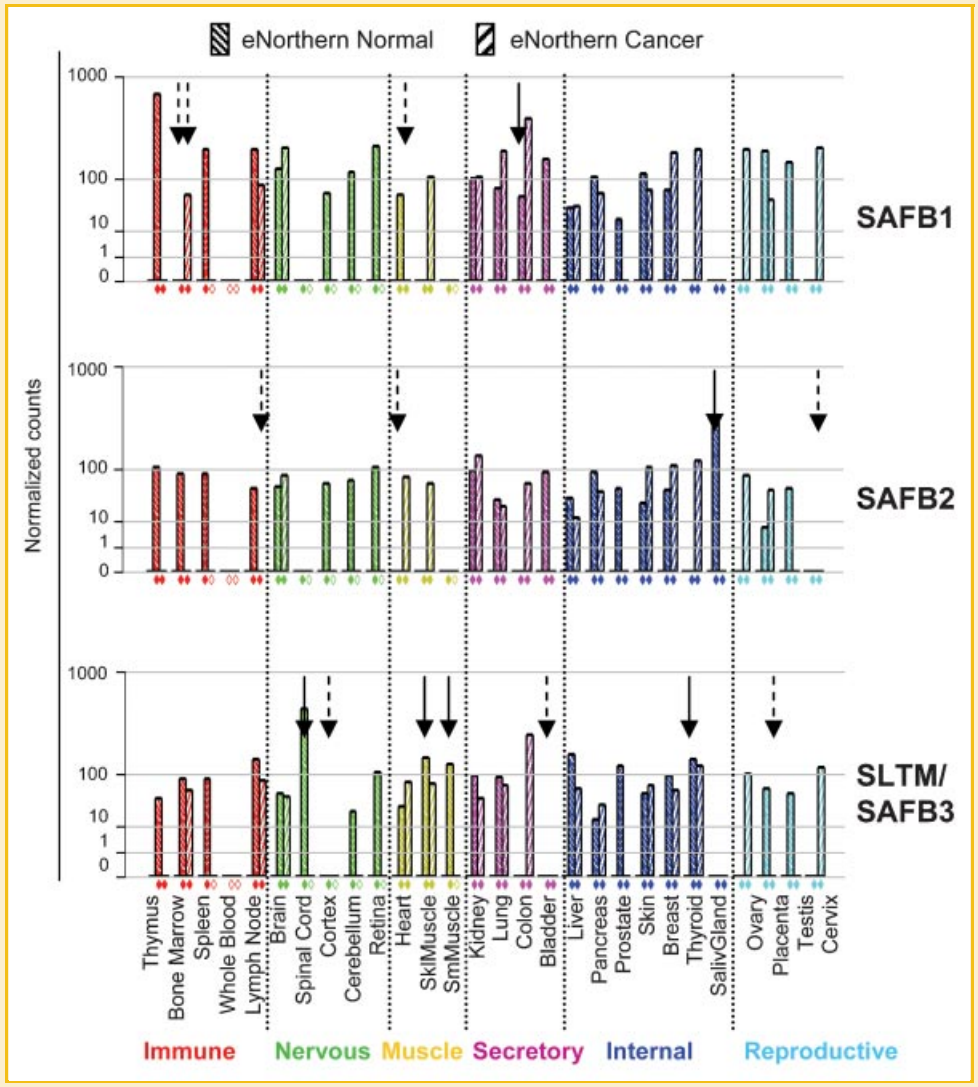

Fig. 2. Expression of SAFB family members. In silico electronic Northern blot data indicate differential expression of SAFB family members in different normal and cancer tissues. Solid arrows indicate unique expression of one family member in a certain tissue. Dashed arrows indicate lack of expression of a family member relative to the other two family members. Data are adapted from www.genecards.org.

SAFB1 regulations the expression of xanthine oxidoreductase (XOR) and sterol regulatory element-binding protein (SREBP)-1c, respectively. SAFB1 represses the expression of XOR, at least in part through interaction with Ku86 and BRG1, which also associate with the XOR promoter [Lin et al., 2008]. XOR plays a role purine metabolism, and is a well-defined source of reactive oxygen species; as such it has been implicated in the development of tissue oxidative damage in a wide variety of respiratory and cardiovascular disorders, as well as in cancer [Bouiez et al., 2008; Lin et al., 2008].

SAFB1 was also shown to regulate the expression of SREBP-1c, a bHLH transcription factor that controls lipogenesis in the liver, and which is induced during overnutrition to facilitate the conversion of glucose to fatty acids and triglycerides for the storage of the excess energy [Omura et al., 2009]. The effect of SAFB1 on SREBP was dependent on RBMX (X chromosome linked RNA binding motif protein) which was previously shown to be important in regulating SREBP-1c expression [Tadashi et al., 2007]. Interestingly, for SREBP-1c, SAFB1 acts to *increase* expression of this gene (in contrast to its well known role in *repression* of transcription). While additional experiments are needed to address this issue, these data clearly suggest that there are gene promoter and cellular context specific factors which may control whether SAFB1 acts to repress or activate gene transcription.

SAFB1 IN RNA SPLICING AND METABOLISM

SAFB1 has been shown to bind to RNA, to interact with RNA processing proteins, and to bind to the C-terminal domain of RNA polymerase II [Nayler et al., 1998a,b; Weighardt et al., 1999; Arao et al., 2000]. Subsequently, SAFB1 was shown to interact and augment the effect of SRrp86 an RNA splicing factor [Li et al., 2003], and to interact with the tissue-specific splicing factor SLM-1 (sam68-like mammalian protein 1) [Stoss et al., 2004]. Together, this

suggests that SAFB1 may be part of a "transcriptosome" complex coupling transcriptional regulation and RNA processing. Further evidence supporting this hypothesis is provided by the finding that SAFB1 interacts with CHD1 a chromatin modifying protein which also possesses activities in RNA splicing [Tai et al., 2003].

The SAFB1 paralog SAFB2 has been shown to be important in formation of alternative RNA splicing complexes [Sergeant et al., 2007] and in the regulation of serine/arginine protein kinases specific for the SR (serine/arginine-rich domain) family of splicing factors, which are thought to play a role in regulation of both constitutive and alternative splicing by regulating intracellular localization of splicing factors [Nikolakaki et al., 2001; Tsianou et al., 2009]. These data suggest a more general role for the SAFB family members in RNA splicing, however this area requires further investigation.

SAFB1—A ROLE IN CHROMATIN ORGANIZATION?

Biochemical experiments have shown that SAFB1 is a nuclear protein which copurifies with chromatin [Renz and Fackelmayer, 1996] and nuclear matrix protein fractions [Oesterreich et al., 1997]. Indeed, SAFB1 binds to AT-rich S/MARs [Nayler et al., 1998a,b] which may provide the basis for higher order chromatin structure and are thought to partition chromatin into distinct topologically independent loops [Bode et al., 1996]. S/MARs have also been shown to be important in modulation of gene expression and in disease processes including cancer [Bode et al., 2000; Gluch et al., 2008]. Similar to S/MARs, base unpairing regions (BURs) are regions in DNA which are of critical importance to higher order chromatin regulation, presumably due their effects regional DNA unwinding and thus enhancement of dynamics accessibility of chromatin [Bode et al., 1992]. We have recently shown that SAFB1 can also bind to BURs, and specifically to multimerized oligonucleotides containing the IgH enhancer fragment (kindly provided by our collaborator Dr. Terumi Kohwi-Shigematsu) (unpublished data). Thus, SAFB1 may function to regulate gene expression though effects on chromatin organization via its binding to S/MARs and BURs. Such role for SAFB1 in chromatin organization is supported by recent work from our group where we showed that SAFB1 is recruited to chromosomal regions harboring gene clusters, for example, the histone gene clusters on chromosomes 1 and 6 [Hammerich-Hille et al., in press]. Since regulation of gene clusters has been shown to involve changes in higher order chromatin structure [as reviewed by Sproul et al., 2005], this finding provides further evidence for a for SAFB1 in chromatin organization.

Further supporting this proposed role for SAFB1 in regulating chromatin architecture, SAFB1 was found to interact with the chromatin remodeling protein CHD1 [Tai et al., 2003]. Although a big and diverse family, common to all chromodomain/helicase/DNA-binding domain (CHD) proteins is their ATP-dependent chromatin-remodeling activity; interestingly, they have also been shown to bind preferentially to AT-rich DNA motifs [reviewed by Marfella and Imbalzano, 2007]. CHD1 also associates with NCoR, and given that SAFB1 has been shown to interact with NCoR [Jiang et al., 2006], it is possible that a CHD1-SAFB1-NCoR repressive complex regulates chromatin structure of at least some SAFB1 target genes.

While the discussed studies provide evidence for a potential role of SAFB1 in regulating chromatin structure, ultimate proof has yet to be provided. While many open questions remain, we would like to speculate that SAFB1 may be a unique protein which can regulate gene expression through dual functions; (i) through classical action in the promoter of target genes, and (ii) through regulation of higher order chromatin structure.

SAFB1 IN CELLULAR PROCESSES

Given the numerous functions of SAFB1, described above, it is of no surprise that it has been implicated in many diverse cellular processes, which we will discuss below.

SAFB1—EFFECTS ON CELL GROWTH AND TRANSFORMATION

SAFB1 functions as a steroid receptor corepressor, it is expressed in various breast cancer cell lines [Townson et al., 2000], and thus an obvious and interesting question was whether it would also regulate growth of breast cancer cells. Overexpression of SAFB1 has proven to be difficult most likely due to toxicity associated with increased levels of SAFB1 protein within the cell. As such, multiple approaches including inducible systems were utilized in attempting to create a cell line with SAFB1 overexpression [Townson et al., 2000]. The small number of colonies with SAFB1 overexpression showed reduced S-phase fraction, reduced cell number, and multi-nuclearity [Townson et al., 2000]. Additional unpublished data from our lab utilizing transient knockdown of SAFB1 by siRNA showed an increase in cell growth confirming that increased SAFB1 levels are associated with growth inhibition.

To further examine cell growth characteristics with loss of SAFB1, we utilized mouse embryonic fibroblasts (MEFs) from SAFB1 germline knockout ($SAFB1^{-/-}$) mice (see discussion below) [Ivanova et al., 2005]. Loss of SAFB1 resulted in lack of senescence and increase of immortalization characteristics [Dobrzycka et al., 2006]. Immortalization of $SAFB1^{-/-}$ MEFs was associated with lack of $p19^{ARF}$ induction and increased levels of TBX2, a T-box transcription factor [Dobrzycka et al., 2006]. Additionally, cells lacking SAFB1 had increased features of a transformed phenotype, including loss of contact inhibition and increased anchorage-independent growth. The results of this study provide further evidence that SAFB1 plays an important role in cellular senescence and immortalization.

Interestingly, SAFB was shown to interact with the zonula occludens protein ZO-2, a member of the membrane-associated guanylate kinase homologue (MAGUK) protein family which is involved in the organization of epithelial and endothelial intercellular junctions [Traweger et al., 2003]. ZO proteins have some unique motifs not shared by other MAGUK family members such as nuclear localization and nuclear export signals. ZO-2 specifically was shown to inhibit proliferation, at least in part through downregulation of cyclin D1 [Huerta et al., 2007; Tapia et al., 2009]. It would be of interest to determine whether this effect was SAFB1 dependent, and whether this interaction is of importance to other SAFB1-mediated functions within the cell.

Collectively, these studies indicate an important function for SAFB1 in cell growth, senescence, and transformation; however, further studies need to be performed to strengthen those findings. For example, it would be of great interest to confirm that the senescence and immortalization characteristics seen in SAFB1 knockout MEFS are recapitulated in human epithelial cells with low SAFB1 levels.

SAFB1 IN STRESS RESPONSE

Heat shock genes are important for the cellular stress response; the original identification of SAFB1 as hsp27-regulating protein suggested a potential link between SAFB1 and cellular stress response, and indeed there is a growing literature supporting this notion. With stress treatment, SAFB1 relocates into nuclear speckles termed stress-induced sub-nuclear bodies (SNBs). These SNBs contain sam68, and are distinct from other described nuclear bodies [Chen et al., 1999; Chiodi et al., 2000]. Stamm's group originally described colocalization of SAFB1 and sam68, with Biamonti's group confirming colocalization of sam68 and SAFB1 in stress induced SNBs [Hartmann et al., 1999; Denegri et al., 2001]. It was also shown that in cells stressed by heat shock, SAFB1 and heat shock factor 1 (HSF1) colocalize in stress-induced SNBs [Weighardt et al., 1999]. This evidence supports the idea that SAFB1 plays an important role in stress response but further experiments are needed to fully comprehend it's role in this process.

ROLE OF SAFB1 IN APOPTOSIS

In addition to its role in cell growth and stress response, SAFB1 has also been shown to be involved in apoptosis. Lee et al. utilized staurosporine (STA) to induce apoptosis, and then examined changes to SAFB1 localization during apoptosis. Prior to treatment, SAFB1 showed a nuclear localization but was excluded from the nucleolus, but shortly after STA treatment, SAFB1 was also found in the nucleolus [Lee et al., 2007]. Thereafter, SAFB1 localized to peri-nucleolar ring structures. The formation of such peri-nucleolar structures was dependent on RNA integrity; however, the SAFB1 localization was not dependent on its RRM or SAFB box, but rather on the C-terminal protein–protein interaction domain.

Similar to other proteins with a direct role in apoptosis, SAFB1 was also shown to be cleaved. Specifically, Lee et al. [2007] showed that it was cleaved in a caspase-3 and SAF-box-dependent manner, after the formation of the peri-nucleolar ring and after cleavage of PARP. Inhibition of caspase-3 lead to a reduction in the number of SAFB1 peri-nucleolar rings suggesting that caspase 3-mediated cleavage of SAFB1 was necessary for the formation of these structures. These data suggest that SAFB1 movement may be associated with maturation of RNA during the initial stages of apoptosis, and that SAFB1 also plays a role in later stages of apoptosis, possibly associated with preparation of DNA for endonuclease-mediated DNA cleavage. While the functional importance of these findings has yet to be discovered and described in more detail, the data clearly suggest a role for SAFB1 in apoptosis. There is again evidence that such function might be shared with other SAFB family members, such as STLM, which as been shown to induce apoptosis—its overexpression resulted in induction of apoptosis, associated with changes in chromatin condensation and cytochrome *c* release [Chan et al., 2007]. It will be of interest whether SAFB2 also plays a role in apoptosis, and if so, by which mechanism.

UNDERSTANDING THE ROLE OF SAFB1 USING MOUSE MODELS

Although exciting, the plethora of functions ascribed to SAFB1, and its involvement in different cellular processes, make it difficult to predict its physiological effects in vivo. We therefore generated a SAFB1$^{-/-}$ mouse line using traditional homologous recombination strategies [Ivanova et al., 2005]. The resulting SAFB1$^{-/-}$ mouse lines have multiple defects including small size and a high degree (although not complete) of lethality [Ivanova et al., 2005]. The breeding (intercrosses) of SAFB1 heterozygous mice (SAFB1$^{+/-}$) produced less than a Mendelian distribution of SAFB1$^{-/-}$ progeny [Ivanova et al., 2005]; detailed examination of genotype distribution during the embryonic and neonatal stages showed prenatal and significant neonatal lethality. This lethality was attributed, at least in part, to defects in lung maturation and in development of the hematopoietic system.

SAFB1 loss also caused significant growth retardation associated with reduced serum IGF-I levels. Defects in the IGF pathway were also seen in SAFB1$^{-/-}$ MEFs which showed increased IGF-I pathway signaling; given the lower IGF-I levels in SAFB1$^{-/-}$ mice, these findings suggest that lack of SAFB1 leads to both systemic but also to cell intrinsic changes in the IGF pathway [Ivanova et al., 2005].

Additionally, both male and female SAFB1$^{-/-}$ mice showed several reproductive defects. Male SAFB1$^{-/-}$ mice were sterile and showed a small reproductive system, were hypogonadal, and had reduced serum testosterone levels. Male SAFB1$^{-/-}$ mice showed progressive tubular degeneration, increased apoptosis of germ cells, and Leydig cell hyperplasia [Ivanova et al., 2005]. Female SAFB1$^{-/-}$ mice were sub-fertile, at least in part due to low serum estradiol and progesterone levels, and defects in oviductal transport. In addition, female SAFB1$^{-/-}$ mice showed secondary infertility due to a loss of follicles associated with increasing atrophy of the ovaries and oviduct [Ivanova et al., 2005].

Another dominant phenotype in the SAFB1$^{-/-}$ mice was a defect in the immune system, clearly recognized by increased signs of infections. Preliminary analysis revealed cutaneous ulcerations, erythemas, increased white blood cell counts, and hypoplasia of the thymus. A further detailed analysis of this phenotype is currently ongoing in our laboratory.

Taken together these data indicate that SAFB1 plays an important role in numerous physiological functions and is especially important in the reproductive system. The pleiotropic defects seen with loss of SAFB1 clearly indicate a lack of redundancy between the SAFB family members raising important additional questions regarding functional relationship between SAFB1, SAFB2, and STLM. They also demand that careful consideration should be undertaken when designing in vivo experiments to discern which phenotype is due to loss of a specific function, or due to systemic defects. For example, considering the known effect of SAFB1 on ER activity, and an association between SAFB levels and breast cancer

outcome (please see below), an analysis of the mammary gland would be of great interest, however, the systemic defects, for example, the low hormone levels, make solid conclusions very difficult, and tissue-specific knock-out mouse models are required.

ASSOCIATION BETWEEN SAFB1 EXPRESSION AND CLINICAL PHENOTYPES

In general, little is known about the role of SAFB1 in human diseases. Earlier studies have implicated SAFB1 in sporadic breast cancer—loss of heterozygosity (LOH) studies showed high rates of up to 78% in microdissected sporadic human breast tumors at marker D19S216 which colocalizes with SAFB1, SAFB2, and other nearby markers at the 19p13 locus [Oesterreich et al., 2001]. This rate of LOH is one the highest described to date. The relevance of this chromosomal region in breast tumorigenesis was confirmed in a large meta-analysis summarizing 151 published LOH studies, totaling >15,000 breast tumors, which identified 19p13 is one of the highest LOH regions in the breast cancer genome [Miller et al., 2003]. Limited sequencing studies have identified some mutation in breast tumors [Oesterreich et al., 2001], providing further evidence for SAFB1 inactivation in breast cancer; this however was not seen in hereditary breast cancer [Bergman et al., 2008]. Previous linkage studies in Swedish families with hereditary breast cancer identified a chromosomal region on 19p where SAFB1 and SAFB2 are located, but direct DNA sequencing and multiplex ligation-dependent probe amplification did not detect any SAFB1 germline frameshift or missense mutations, and large deletions or amplifications, respectively [Bergman et al., 2007]. These data indicate that SAFB1 is not involved in hereditary breast cancer but may be important in sporadic breast cancer. This is supported by finding that loss of SAFB1/SAFB2 protein expression, as measured by immunoblotting with a pan-SAFB antibody, was associated with significantly worse survival of breast cancer patients who did not receive adjuvant therapy (surgery only) [Hammerich-Hille et al., 2009]. Multi-variate analysis revealed negative ERα status, more than three positive lymph nodes, and low SAFB were all associated with increased risk of recurrence. There was no correlation between SAFB1/SAFB2 levels and outcome in tamoxifen-treated patients, suggesting that the SAFB association with a more aggressive tumor phenotype and worse patient outcomes was not ER related, but due to one of the other discussed SAFB functions.

It is of interest to note that SATB1 (special AT-rich binding protein 1) was recently shown to promote breast tumor growth and metastasis through reprogramming gene expression in the cells [Han et al., 2008]. Besides the similarity in names (which is unfortunate since it can obviously cause confusion among researchers!), it seems that the two proteins share some other properties. SAFB1 and SATB1 are highly expressed in the immune system; indeed SATB1 was originally described as a thymocyte-specific protein, and was shown to regulate gene expression in T-cells [Dickinson et al., 1992]. Just like SAFB1$^{-/-}$ mice, the SATB1-null mice are small in size, have disproportionately small thymuses and spleens, and show high degrees of lethality [Alvarez et al., 2000]. Both proteins can bind to BURs, and SATB1 also has a highly related family member, called SATB2 [Dobreva et al., 2003]. Similar to what has been described for SAFB1 [Lee et al., 2007], SATB1 also undergoes caspase-mediated cleavage during apoptosis [Galande et al., 2001; Sun et al., 2006; Tan et al., 2008]. In future studies it would be of interest to determine whether SAFB1 and SATB1 directly interact, and possibly synergize in some functions.

Finally, as shown in Figure 2 and discussed above, SAFB1 is highly expressed in many other tissues such as brain, skin, and lymph nodes, and there is differential expression between normal and tumor tissues in many organs such as thymus, prostate, and cervix, and we might therefore see additional yet to be described roles of SAFB1 in other diseases, including tumorigenesis in other tissues.

SUMMARY AND CONCLUSIONS

SAFB1 is a large multi-functional protein which is involved in numerous cellular processes. Research has indicated a role for SAFB1 in transcriptional regulation, RNA splicing and metabolism, and more recent data suggest a role in chromatin organization, and we are just beginning to understand how these functions are connected to its role in various cellular processes, such as cell growth, stress response and apoptosis (see Fig. 3).

SAFB1 also acts to transcriptionally repress the activity of ERα one of the most critical players in breast tumorigenesis. It functions

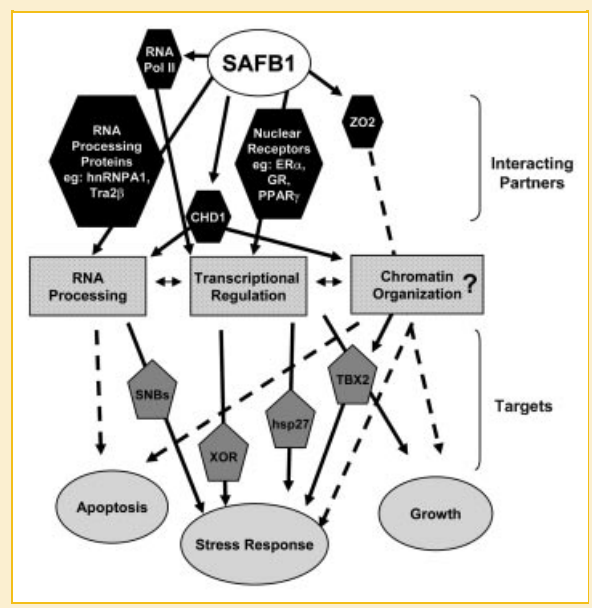

Fig. 3. Model of SAFB1 as a multi-functional protein. Rectangles indicate (known, and yet to be proven) functions of SAFB1, and ovals indicate the cellular processes SAFB1 is involved in. Hexagons indicate SAFB1 interacting partners which may provide links to SAFB1's functions or role in cellular processes. Pentagons represent those proteins which are downstream targets of SAFB1 functions which may link function to cellular process. Solid arrows indicate previously demonstrated relationships and dashed arrows are potential relationships not yet proven. This model indicates the complexity of SAFB1 in linking interacting partners, functions of SAFB1, downstream SAFB1 targets, and cellular processes.

as a growth inhibitor in breast cancer cells, and maps to chromosome 19p13 which displays one of the highest rates of LOH reported in breast cancer. Association of SAFB1 with clinical biomarkers and survival data provide convincing evidence that SAFB1 is involved in breast tumorigenesis, though the extent of involvement based on current knowledge is not completely understood.

Although the described studies show SAFB1's involvement in many functions and cellular processes, many open questions remain. For example, unpublished data from our group have indicated potential roles for SAFB1 in DNA surveillance and in replication, but details are yet to be elucidated. We need to know more about the interplay of SAFB1 with the other SAFB family members. While SAFB2 and SLTM share regions of high homology with SAFB1, there does not appear to be functional redundancy between the family members. It is an open question in which functions and cellular processes SAFB1, SAFB2, and STLM functions additive, synergistic, or potentially antagonistic.

In summary—"Lots Still to Be Done"! Only the further detailed study of SAFB1 and its related proteins, using new approaches, and models such as tissue-specific knockout mice, will fully reveal its functions and their involvement in normal physiology and disease.

ACKNOWLEDGMENTS

The SAFB-related research in Dr Oesterreich's Lab has been funded by NIH grants R01 CA097213 and P01030195 (SO). Jason Garee received a Department of Defense Breast Cancer Research Program grant (W81XWH-06-1-0710).

REFERENCES

Alvarez J, Yasui D, Niida H, Joh T, Loh D, Kohwi-Shigematsu T. 2000. The MAR-binding protein SATB1 orchestrates temporal and spatial expression of multiple genes during T-cell development. Genes Dev 14:521–535.

Arao Y, Kuriyama R, Kayama F, Kato S. 2000. A nuclear matrix-associated factor, SAF-B, interacts with specific isoforms of AUF1/hnRNP D. Arch Biochem Biophys 380(2):228.

Bergman A, Abel F, Behboudi A, Yhr M, Mattsson J, Svensson J, Karlsson P, Nordling M. 2008. No germline mutations in supposed tumour suppressor genes SAFB1 and SAFB2 in familial breast cancer with linkage to 19p. BMC Med Genet 9(1):108.

Bergman A, Karlsson P, Berggren J, Martinsson T, Bjorck K, Nilsson S, Wahlstrom J, Wallgren A, Nordling M. 2007. Genome-wide linkage scan for breast cancer susceptibility loci in Swedish hereditary non-*BRCA1/2* families: Suggestive linkage to 10q23.32-q25.3. Genes Chromosomes Cancer 46:302–309.

Bode J, Benham C, A K, C M. 2000. Transcriptional augmentation: Modulation of gene expression by scaffold/matrix-attached regions (S/MAR elements). Crit Rev Eukaryot Gene Expr 10:73–90.

Bode J, Kohwi Y, Dickinson L, Joh T, Klehr D, Mielke C, Kohwi-Shigematsu T. 1992. Biological significance of unwinding capability of nuclear matrix-associating DNAs. Science 255(5041):195–197.

Bode J, Stengert-Iber M, Kay V, Schlake T, Dietz-Pfeilstetter A. 1996. Scaffold/matrix-attached regions: Topological switched with multiple regulatory functions. Crit Rev Eukaryot Gene Expr 6:115–138.

Boueiz A, Damarla M, Hassoun PM. 2008. Xanthine oxidoreductase in respiratory and cardiovascular disorders. Am J Physiol Lung Cell Mol Physiol 294(5):L830–L840.

Chan CW, Lee Y-B, Uney J, Flynn A, Tobias JH, Norman M. 2007. A novel member of the SAF (scaffold attachment factor)-box protein family inhibits gene expression and induces apoptosis. Biochem J 407(3):355–362.

Chen T, Boisvert F-M, Bazett-Jones DP, Richard S. 1999. A role for the GSG domain in localizing Sam68 to novel nuclear structures in cancer cell lines. Mol Biol Cell 10(9):3015–3033.

Chiodi I, Biggiogera M, Denegri M, Corioni M, Weighardt F, Cobianchi F, Riva S, Biamonti G. 2000. Structure and dynamics of hnRNP-labelled nuclear bodies induced by stress treatments. J Cell Sci 113(22):4043–4053.

Debril MB, Dubuquoy L, Feige JN, Wahli W, Desvergne B, Auwerx J, Gelman L. 2005. Scaffold attachment factor B1 directly interacts with nuclear receptors in living cells and represses transcriptional activity. J Mol Endocrinol 35(3):503–517.

Denegri M, Chiodi I, Corioni M, Cobianchi F, Riva S, Biamonti G. 2001. Stress-induced nuclear bodies are sites of accumulation of pre-mRNA processing factors. Mol Biol Cell 12(11):3502–3514.

Dickinson LA, Joh T, Kohwi Y, Kohwi-Shigematsu T. 1992. A tissue-specific MAR SAR DNA-binding protein with unusual binding site recognition. 70(4):631–645.

Dobreva G, Dambacher J, Grosschedl R. 2003. SUMO modification of a novel MAR-binding protein, SATB2, modulates immunoglobin m gene expression. Genes Dev 17:3048–3061.

Dobrzycka KM, Kang K, Jiang S, Meyer R, Rao PH, Lee AV, Oesterreich S 2006. Disruption of Scaffold Attachment Factor B1 Leads to TBX2 Up-regulation, Lack of p19ARF Induction, Lack of Senescence, and Cell Immortalization. Cancer Res 66(16):7859–7863.

DuPont B, Garcia D, Sullivan T, Naylor S, Oesterreich S. 1997. Assignment of SAFB encoding Hsp27 ERE-TATA binding protein (HET)/scaffold attachment factor B (SAF-B) to human chromosome 19 band p13. Cytogenet Cell Genet 79:284–285.

Galande S, Dickinson LA, Mian IS, Sikorska M, Kohwi-Shigematsu T. 2001. SATB1 cleavage by caspase 6 disrupts PDZ domain-mediated dimerization, causing detachment from chromatin early in T-cell apoptosis. Mol Cell Biol 21(16):5591–5604.

Gluch A, Vidakovic M, Bode J. 2008. Scaffold/matrix attachment regions (S/MARs): Relevance for disease and therapy. Protein-Protein Interactions as New Drug Targets:67–103.

Hammerich-Hille S, Bardout V, Hilsenbeck S, Osborne C, Oesterreich S. 2009. Low SAFB levels are associated with worse outcome in breast cancer patients. Breast Cancer Res Treat [Epub ahead of print].

Hammerich-Hille S, Kaipparettu BA, Tsimelzon A, Creighton CJ, Jiang S, Polo JM, Melnick A, Meyer R. In press. Oesterreich S SAFB1 mediates repression of immune regulators and apoptotic genes in breast cancer cells. J Biol Chem [In Press].

Han H-J, Russo J, Kohwi Y, Kohwi-Shigematsu T. 2008. SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis. Nature 452:187–193.

Hartmann AM, Nayler O, Schwaiger FW, Obermeier A, Stamm S. 1999. The interaction and colocalization of Sam68 with the splicing-associated factor YT521-B in nuclear dots is regulated by the Src family kinase p59fyn. Mol Biol Cell 10(11):3909–3926.

Huerta M, Munoz R, Tapia R, Soto-Reyes E, Ramirez L, Recillas-Targa F, Gonzalez-Mariscal L, Lopez-Bayghen E. 2007. Cyclin D1 is transcriptionally down-regulated by ZO-2 via an E Box and the transcription factor c-Myc. Mol Biol Cell 18(12):4826–4836.

Ivanova M, Dobrzycka KM, Jiang S, Michaelis K, Meyer R, Kang K, Adkins B, Barski OA, Zubairy S, Divisova J, Lee AV, Oesterreich S. 2005. Scaffold attachment factor B1 functions in development, growth, and reproduction. Mol Cell Biol 25(8):2995–3006.

Jiang S, Meyer R, Kang K, Osborne CK, Wong J, Oesterreich S. 2006. Scaffold attachment factor SAFB1 suppresses estrogen receptor {alpha}-mediated

transcription in part via interaction with nuclear receptor corepressor. Mol Endocrinol 20(2):311–320.

Kipp M, Gohring F, Ostendorp T, van Drunen CM, van Driel R, Przybylski M, Fackelmayer FO. 2000. SAF-box, a conserved protein domain that specifically recognizes scaffold attachment region DNA. Mol Cell Biol 20(20):7480–7489.

Lee Y-B, Colley S, Norman M, Biamonti G, Uney JB. 2007. SAFB redistribution marks steps of the apoptotic process. Exp Cell Res 313(18):3914.

Li J, Hawkins IC, Harvey CD, Jennings JL, Link AJ, Patton JG. 2003. Regulation of alternative splicing by SRrp86 and its interacting proteins. Mol Cell Biol 23(21):7437–7447.

Lin J, Xu P, LaVallee P, Hoidal JR. 2008. Identification of Proteins Binding to E-Box/Ku86 Sites and Function of the Tumor Suppressor SAFB1 in Transcriptional Regulation of the Human Xanthine Oxidoreductase Gene. Journal of Biological Chemistry 283:29681–29689.

Lonard DM, Lanz RB, O'Malley BW. 2007. Nuclear receptor coregulators and human disease. Endocr Rev 28(5):575–587.

Lonard DM, O'Malley BW. 2007. Nuclear receptor coregulators: Judges, juries and executioners of cellular regulation. Mol Cell 27:691–700.

Marfella CGA, Imbalzano AN. 2007. The Chd family of chromatin remodelers. Mutat Res 618(1–2):30.

Miller BJ, Wang D, Krahe R, Wright FA. 2003. Pooled analysis of loss of heterozygosity in breast cancer: A genome scan provides comparative evidence for multiple tumor suppressors and identifies novel candidate regions. Am J Hum Genet 73(4):748–767.

Nayler O, Stratling W, Bourquin J, Stagljar I, Lindemann L, Jasper H, Hartmann A, Fackelmayer F, Ullrich A, Stamm S. 1998b. SAF-B protein couples transcription and pre-mRNA splicing to SAR/MAR elements. Nucleic Acids Res 26(15):3542–3549.

Nayler O, Stratling W, Bourquin JP, Stagljar I, Lindemann L, Jasper H, Hartmann AM, Fackelmayer FO, Ullrich A, Stamm S. 1998b. SAF-B protein couples transcription and pre-mRNA splicing to SAR/MAR elements. Nucl Acids Res 26:3542–3549.

Nikolakaki E, Kohen R, Hartmann AM, Stamm S, Georgatsou E, Giannakouros T. 2001. Cloning and characterization of an alternatively spliced form of SR protein kinase 1 that interacts specifically with scaffold attachment factor-B. J Biol Chem 276:40175–40182.

Oesterreich S. 2003. Scaffold attachment factors SAFB1 and SAFB2: Innocent bystanders or critical players in breast tumorigenesis? J Cell Biochem 90(4):653–661.

Oesterreich S, Allredl DC, Mohsin SK, Zhang Q, Wong H, Lee AV, Osborne CK, O'Connell P. 2001. High rates of loss of heterozygosity on chromosome 19p13 in human breast cancer. Br J Cancer 84(4):493.

Oesterreich S, Lee A, Sullivan T, Samuel S, Davie J, Fuqua SAW. 1997. Novel nuclear matrix protein HET binds to and influences activity of the HSP27 promoter in human breast cancer cells. J Cell Biochem 67(2):275–286.

Oesterreich S, Zhang Q, Hopp T, Fuqua SAW, Michaelis M, Zhao HH, Davie JR, Osborne CK, Lee AV. 2000a. Tamoxifen-bound estrogen receptor (ER) strongly interacts with the nuclear matrix protein HET/SAF-B, a novel inhibitor of ER-mediated transactivation. Mol Endocrinol 14(3):369–381.

Oesterreich S, Zhang QP, Lee AV. 2000b. Inhibition of oestrogen receptor activity by the co-repressor HET/SAF-B is relieved by blockade of histone deacetylase activity. Eur J Cancer 36(Supplement 4):43.

Omura Y, Nishio Y, Takemoto T, Ikeuchi C, Sekine O, Morino K, Maeno Y, Obata T, Ugi S, Maegawa H, Kimura H, Kashiwagi A. 2009. SAFB1, an RMBX-binding protein, is a newly identified regulator of hepatic SREBP-1c gene. BMB Reports 42:232–237.

Renz A, Fackelmayer FO. 1996. Purification and molecular cloning of the scaffold attachment factor B (SAF-B), a novel human nuclear protein that specifically binds to S/MAR-DNA. Nucleic Acids Res 24(5):843–849.

Sergeant KA, Bourgeois CF, Dalgliesh C, Venables JP, Stevenin J, Elliott DJ. 2007. Alternative RNA splicing complexes containing the scaffold attachment factor SAFB2. J Cell Sci 120(2):309–319.

Sproul D, Gilbert N, Bickmore WA. 2005. The role of chromatin structure in regulating the expression of clustered genes. Nat Rev Genet 6(10):775.

Stoss O, Novoyatleva T, Gencheva M, Olbrich M, Benderska N, Stamm S. 2004. p59fyn-mediated phosphorylation regulates the activity of the tissue-specific splicing factor rSLM-1. Mol Cell Neurosci 27(1):8–21.

Sun Y, Wang T, Su Y, Yin Y, Xu S, Ma C, Han X. 2006. The behavior of SATB1, a MAR-binding protein, in response to apoptosis stimulation. Cell Biol Int 30(3):244–247.

Tadashi T, Yoshihiko N, Osamu S, Chikako I, Yoshio N, Yasuhiro M, Hiroshi M, Hiroshi K, Atsunori K. 2007. RBMX is a novel hepatic transcriptional regulator of SREBP-1c gene response to high-fructose diet. FEBS Lett 581(2):218–222.

Tai HH, Geisterfer M, Bell JC, Moniwa M, Davie JR, Boucher L, McBurney MW. 2003. CHD1 associates with NCoR and histone deacetylase as well as with RNA splicing proteins. Biochem Biophys Res Commun 308(1):170.

Tan J, Sun Y, Song J, Chen Y, Krontiris T, Durrin L. 2008. SUMO conjugation to the matrix attachment region-binding protein, special AT-rich sequence-binding-protein-1 (SATB1), targets SATB1 to promyelocytic nuclear bodies where it undergoes caspase cleavage. J Biol Chem 283:18124–18134.

Tapia R, Huerta M, Islas S, Avila-Flores A, Lopez-Bayghen E, Weiske J, Huber O, Gonzalez-Mariscal L. 2009. Zona occludens-2 inhibits cyclin D1 expression and cell proliferation and exhibits changes in localization along the cell cycle. Mol Biol Cell 20(3):1102–1117.

Townson SMDK, Lee AV, Air M, Deng W, Kang K, Jiang S, Kioka N, Michaelis K, Oesterreich S. 2003. SAFB2, a new scaffold attachment factor homolog and estrogen receptor corepressor. J Biol Chem 278:20059–20068.

Townson SMKK, Lee AV, Oesterreich S. 2004. Structure-function analysis of the estrogen receptor alpha corepressor scaffold attachment factor B1: Identification of a potent transcriptional repression domain. J Biol Chem 279:26074–26081.

Townson SMST, Zhang Q, Clark GM, Osborne CK, Lee AV, Oesterreich S. 2000. HET/SAF-B overexpression causes growth arrest and multinuclearity and is associated with aneuploidy in human breast cancer. Clin Cancer Res 6:3788–3796.

Traweger AFR, Krizbai IA, Weiger TM, Bauer H-C, Bauer H. 2003. The tight junction protein ZO-2 localizes to the nucleus and interacts with the heterogeneous nuclear ribonucleoprotein scaffold attachment factor-B. J Biol Chem 278:2692–2700.

Tsianou D, Nikolakaki E, Tzitzira A, Bonanou S, Giannakouros T, Georgatsou E. 2009. The enzymatic activity of SR protein kinases 1 and 1a is negatively affected by interaction with scaffold attachment factors B1 and 2. FEBS J 276(18):5212–5227.

Weighardt F, Cobianchi F, Cartegni L, Chiodi I, Villa A, Riva S, Biamonti G. 1999. A novel hnRNP protein (HAP/SAF-B) enters a subset of hnRNP complexes and relocates in nuclear granules in response to heat shock. J Cell Sci 112(10):1465–1476.