Transcriptional Activation by a Matrix Associating 
Region-binding Protein

CONTEXTUAL REQUIREMENTS FOR THE FUNCTION OF BRIGHT*

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Bright (B cell regulator of IgH transcription) is a B cell-specific, matrix associating region-binding protein that transactivates gene expression from the IgH intronic enhancer (Eμ). We show here that Bright has multiple contextual requirements to function as a transcriptional activator. Bright cannot transactivate via out of context, concatenated binding sites. Transactivation is maximal on integrated substrates. Two of the three previously identified binding sites in Eμ are required for full Bright transactivation. The Bright DNA binding domain defined a new family, which includes SWI1, a component of the SWI/SNF complex shown to have high mobility group-like DNA binding characteristics. Similar to one group of high mobility group box proteins, Bright distorts Eμ binding site-containing DNA on binding, supporting the concept that it mediates Eμ remodeling. Transfection studies further implicate Bright in facilitating spatially separated promoter-enhancer interactions in both transient and stable assays. Finally, we show that overexpression of Bright leads to enhanced DNase I sensitivity of the endogenous Eμ matrix associating regions. These data further suggest that Bright may contribute to increased gene expression by remodeling the immunoglobulin locus during B cell development.

Transcriptional regulation of genes during development and differentiation is tightly controlled through several mechanisms. The tissue specificity conferred by the immunoglobulin heavy chain enhancer (Eμ)† has been studied extensively both for understanding Ig regulation and as a model for enhancer function (reviewed in Ref. 1). Eμ is a complex unit containing binding sites for multiple transcription factors and can functionally be broken down into two segments, the core and the flanking matrix associating regions (MARs) (2–5). Most of the previously identified factors bind to the enhancer core, and several have been shown to have some B cell specificity in terms of expression or ability to transactivate. However, no binding site in isolation can confer all of the tissue-specific regulation seen in vivo. Ultimately, this is the result of cumulative interactions of various nuclear factors with both DNA and each other. The Eμ core segment alone can increase transcription in transient systems (6, 7). In vivo, however, the core alone is insufficient to drive transcription or maintain tissue specificity. Transgenic studies have demonstrated that high level tissue-specific expression is only seen when the core is present in context of the MARs (8). This effect requires the core, because MARs alone could not produce high level expression. Although the MARs had previously been implicated in negative regulation of the Ig locus in non-B cells (4, 9–12), this was the first demonstration that the MARs were required for proper expression in B cells.

Bright (B cell regulator of IgH transcription) is the only B cell-specific transcription factor shown to bind to, and transactivate via, the Eμ MARs (13). Bright was first identified as a factor responsible for increased expression of the immunoglobulin heavy chain gene following antigen + interleukin 5 stimulation of B cells in culture (14, 15). The Bright binding complex has also recently been shown to contain Btk, which is critical for the DNA binding complex (16). Bright binds within the MARs of the IgH enhancer to distinct ATC motifs (P sites) previously identified as binding sites for the Eμ negative regulator, nuclear factor-κ B, negative regulator, and the MAR-binding protein, SATB1 (11, 17). We have identified nuclear factor-κ B, negative regulator as a previously characterized, lineage-non-restricted homeoprotein, Cux/CAAT displacement protein (18), that antagonizes Bright binding and transactivation by direct competition for P sites. Developmentally, Bright expression is maximal in late stage B cells (13, 19), a pattern opposite that of Cux/CAAT displacement protein (18). Bright is found in the nuclear matrix and within matrix-associated PML nuclear bodies (13, 20), locations consistent with a putative role in chromosomal organization. Although a number of MAR binding factors have been cloned (e.g. see Refs. 13, 17, and 21–29, and reviewed in Ref. 30), Bright was the first shown to directly affect gene transcription.

MARs and attachment to the nuclear matrix can mediate specific alterations in chromatoin structure (31–34). Such a mechanism seemed reasonable for Bright, based on features of its DNA binding. Highly specific binding within the minor groove is achieved by virtue of two domains (reviewed in Ref.
a self-association/tetramerization domain, termed REKLES for a heptapeptide conserved within this region among Bright orthologues, and a DNA binding region, termed ARID for AT-rich interaction domain. The Bright ARID defines a new family of DNA-binding proteins, including SWI1, a component of the SWI-SNF complex that has been shown to remodel chromatin (36) and p270, its apparent mammalian orthologue (37). Components of human SWI-SNF appear to be tightly associated with the nuclear matrix (38), suggesting that at least a fraction of this complex could be involved in chromatin organizational properties associated with MARs (reviewed in Ref. 39). Like SWI-SNF, all ARID proteins bind AT-rich DNA, but only members that contain both ARID and REKLES bind specifically to AT-rich MAR motifs (35).

In this report we further characterize the mechanisms through which Bright functions and the contextual requirements for Bright transactivation. We also show that Bright bends its DNA target on binding. This, along with the observation that Bright overexpression induces increased DNAse I hypersensitivity of the enhancer, provides a rationale for how this protein may facilitate enhanced expression of the immunoglobulin gene.

**EXPERIMENTAL PROCEDURES**

** Constructs — The derivation of $\Delta E_\rho$ and $\Delta E_\mu$ mutants was described previously (13). $E_\mu$ and $\Delta E_\mu$ were cloned in the XbaI site of the pBL-CAT2 vector. All ARID mutants were cloned in the Sall-BamHI sites of the CAT vector. The hybrid SV40-MAR construct (40) was previously constructed. The S107 promoter isolator was a BamHI-HaeIII fragment (covering nucleotides 550 to 40), blunt-ended, and cloned into the pBL-CAT2 vector. Vectors containing elements distal of the cat gene were constructed by first subcloning $E_\mu$ of the appropriate mutation into pBluescript (Stratagene) and cloning a $KpnI$-SacI fragment into the distal site of either pBL-CAT2 or pBL-CAT2 containing the S107 promoter fragment.

**Electrophoretic Mobility Shift Assay — Specifics of binding reactions were described previously (13). To assess binding to the four P sites, the following constructs were used: the $P2 E_\mu$ 5' MAR isolates the P1 site, the $P3 E_\mu$ 5' MAR isolates the P2 site, the $P4 E_\mu$ 3' MAR isolates the P3 site, and the $P3 E_\mu$ 3' MAR isolates the P4 site. Briefly, these fragments were end-labeled (20,000 cpm) with [32P]dCTP and bound to nuclei isolated Bright protein in the presence of increasing concentrations of poly(d-I-C), and run on a 4% nondenaturing polyacrylamide gels. Gels were dried and exposed to x-ray film.**

**Transfections and Stable Lines — Transfections of M12.4 and J558L cells and analysis of CAT protein was done as described previously (13). Stable transfected lines were made by co-transfecting the indicated CAT vectors with a 3-fold excess to pBR-cytomegalovirus (Stratagene). 48 h after transfection, cells were selected in G418. Transient transfection of Bright to transactivate gene expression from a binding site not in context of the $E_\mu$ enhancer, we used reporter constructs containing catamers of a binding site in the S107 promoter (Bf150) or the $E_\mu$ P2 site in transient transfections. Expression constructs containing Bright in either the sense or antisense orientation were co-transfected with reporter constructs driven by a thymidine kinase promoter and the additional elements as described in Fig. 1. Concatamers of the P2 site, which gel shift analysis demonstrated is a strong Bright binding site, could not increase CAT levels in either a B cell or plasma cell line (Fig. 1). Similarly, a reporter construct with the S107 MAR site containing an IgH enhancer element (E$\mu$) upstream of a reporter gene. To assess the ability of Bright to transactivate gene expression from a binding site not in context of the $E_\mu$ enhancer, we used reporter constructs containing catamers of a binding site in the S107 promoter (Bf150) or the $E_\mu$ P2 site in transient transfections. Expression constructs containing Bright in either the sense or antisense orientation were co-transfected with reporter constructs driven by a thymidine kinase promoter and the additional elements as described in Fig. 1. Concatamers of the P2 site, which gel shift analysis demonstrated is a strong Bright binding site, could not increase CAT levels in either a B cell or plasma cell line (Fig. 1). Similarly, a reporter construct with the S107 MAR site catamerized to eight repeats (Bf150 × 8) did not show any significant increase in transcription when Bright was co-transfected in the sense orientation.**

**RESULTS**

*Bright Does Not Transactivate from a Concatamerized Binding Site — In our first description of the Bright transcription factor (13), we demonstrated that Bright could transactivate gene expression from a plasmid containing an IgH enhancer element ($E_\mu$) upstream of a reporter gene. To assess the ability of Bright to transactivate gene expression from a binding site not in context of the $E_\mu$ enhancer, we used reporter constructs containing catamers of a binding site in the S107 promoter (Bf150) or the $E_\mu$ P2 site in transient transfections. Expression constructs containing Bright in either the sense or antisense orientation were co-transfected with reporter constructs driven by a thymidine kinase promoter and the additional elements as described in Fig. 1. Concatamers of the P2 site, which gel shift analysis demonstrated is a strong Bright binding site, could not increase CAT levels in either a B cell or plasma cell line (Fig. 1). Similarly, a reporter construct with the S107 MAR site catamerized to eight repeats (Bf150 × 8) did not show any significant increase in transcription when Bright was co-transfected in the sense orientation.**

*Bright Requires Specific MAR Sequences for Transactivation Function — Despite the lack of Bright activity on a concatamerized substrate, Bright clearly activated transcription from an $E_\mu$ element over the levels seen from $E_\mu$ alone (see Ref. 13 and Fig. 2). Bright binding sites were required for this activity, because an $E_\mu$ that lacked the P sites ($\Delta E_\mu$) did not mediate Bright transactivation (see Ref. 13 and Fig. 2). To further examine the specificity for transactivation that $E_\mu$ ascribes to Bright, we tested the effects of P site deletions. Because $P2$ is a well characterized Bright binding site (13), we reasoned that it might be capable of acting alone. Indeed, a construct that lacks P1, P3, and P4 (AP1, P3, P4) was competent in mediating

**Fig. 1. Bright does not transactivate from a concatamerized binding site. M12.4 or J558L cells were transfected with tk-CAT vectors containing catamers of the $E_\mu$ P2 site or of the high affinity Bf150 site from the S107 promoter as indicated. Cells were co-transfected with an expression vector containing Bright in the sense (filled bars) or antisense (open bars) orientation. Results are expressed as fold activation over tk-CAT alone and are the average of at least four separate experiments.**
Bright transactivation (Fig. 2). However, a construct that lacked the P2 but had all other sites intact (ΔP2) was still functional. The additional deletion of the P4 site (ΔP2, P4) abrogated Bright-mediated function. That P4 could mediate Bright transactivation alone was verified using a P4-only construct (ΔP1, P2, P3). Interestingly, there was a trend that the P2-only and P4-only constructs were activated to a slightly lower degree than the intact Eμ, though the difference was not statistically significant. It seems possible that Bright can act through both sites but that the activity seen in the intact Eμ may be the combined effects of Bright binding to both sites. It was unanticipated that Bright could not function through the P3 site, because Bright also binds P3 very well (Fig. 3). This lack of function suggested that competent Bright binding sites must be within a contextual arrangement to allow them to mediate transactivation.

Bright Mediates Promoter-Enhancer Interactions—Knowing that Bright could mediate transactivation from both the enhancer and the S107 promoter (and possibly other Ig promoters, as well), we became interested in determining whether these functions were independent or whether these elements could function in concert. We constructed CAT vectors that partially or completely recapitulated the immunoglobulin locus promoter/enhancer arrangement. The S107 promoter fragment contains two Bright binding sites, one of which functions as a MAR (15, 45). In a construct containing the promoter alone, Bright could not transactivate in a transient assay (Fig. 4). This is in contrast to assays where Eμ is placed 5’ of the CAT gene and Bright effectively increased gene expression. The ability of Bright to function through the IgH enhancer is also seen when the enhancer is in the distal position. Strikingly, when both the promoter and enhancer are present in the same construct, the effects of Bright are synergistic, increasing transcription levels more than 3-fold over that seen with Eμ alone in the distal position. This Bright-mediated transactivation requires Bright binding, because a construct with ΔEμ in the distal position could not mediate the Bright effect (Fig. 4).

Bright Transactivates Integrated Targets by MAR Interaction—Because Bright binding sites have the potential to act as MARs, we also studied these vectors in stably transfected cells to determine whether Bright can mediate MAR effects that would only be detected from integrated targets. CAT constructs were stably transduced into J558L cells and selected with neomycin for 21 days before transient transfection with Bright sense or antisense constructs. In contrast to results from the transient transfection assay, Bright is able to transactivate from the promoter alone in the stable system (Fig. 4). This supports a role for Bright as a MAR-binding protein, because this phenomenon is only seen when the promoter construct is integrated into the chromosome. A further increase in S107 promoter-driven transcription is seen when Eμ is present in the distal position. As in the transient studies, this interaction is specific for Bright binding, because a construct with ΔEμ in the distal position does not transactivate beyond what is seen with promoter alone (Fig. 4).

Bright Mediates DNA Distortion—The distance between promoter-associated and enhancer-associated Bright sites that appear to synergize in the constructs of Fig. 4 is about 2 kilobase pairs. We assumed that Bright may affect DNA topology to facilitate these interactions. We have previously shown that Bright binds DNA in the minor groove (13). The class of high mobility group box proteins typified by lymphoid enhancer-binding factor-1 and SRY bind DNA in the minor groove and bend the double helix (41). To determine whether Bright can also distort its DNA target on binding, we used the circular permutation assay described by Giese et al. (41), which measures DNA bending, as well as DNA flexibility caused by changes in DNA structure such as melting of AT-rich regions. For this assay, a series of equally sized fragments, differing only in the position of a Bright binding site, were generated. If the DNA is distorted during binding, then fragments bound near the center will migrate through a gel at a slower rate than those bound near the ends. In Fig. 5, a truncated Bright protein (amino acids 216–601) with full binding activity distorts the circular permuted fragments as assessed by differences in complex mobility. The full-length Bright protein had an identical effect in this assay (data not shown). The angle of induced distortion can be determined by comparing the calculated ratio $\mu M/\mu E$ to a plot of known A-tract standards, where $\mu M$ and $\mu E$ are the relative mobilities of the middle-bound (slowest migrating) and end-bound (fastest migrating) fragments, respectively (42). For Bright, $\mu M$ is calculated to be 0.41 and $\mu E$ to 0.48 giving a ratio of 0.85. Based on A-tract standards in 4% polyacrylamide gels, this ratio corresponds to a distortion angle of 80–90°.

$E\mu$ Becomes DNase I Hypersensitive following Bright Over-expression—The ability of Bright to mediate specific activation of integrated binding sites and to distort DNA suggested that it may be involved with altering chromosomal architecture and nucleosome-free regions of DNA. DNase I hypersensitive sites coincide with nucleosome-free regions in chromatin. To test the ability of Bright to alter the chromosomal organization of the endogenous IgH locus, we stably transfected Bright into a murine mature B cell line (WEHI 231) that produces low levels of endogenous Bright protein (13). Following a 20-day culture in G418, we selected a clone that expressed Bright at levels ~8-fold above that in the WEHI 231 parental lane and about twice that seen in two IgM-secreting plasmacytomas (MOPC 104E and HNK-1; data not shown). Nucleosome sensitivity in mock-transfected WEHI 231 nuclei was limited to a 220-bp region coinciding with the $E\mu$ core (Fig. 6). In cells ectopically expressing Bright, hypersensitivity was greater in magnitude and encompassed a significantly larger (~500 bp) area that extended through the 5′ MAR, which contains the high affinity P2 binding site of Bright. A modest (2–3-fold) increase in $\mu$ transcription accompanied this effect (data not shown) but is similar to the level of $\mu$ induction caused by antigen + inter-

![Figure 2](https://example.com/fig2.png)
leukin 5 stimulation (14, 15). A stronger and more extended DNase I digestion pattern is observed (Fig. 6) in nuclei of the plasmacytomas that transcribe the $\mu$ locus about 50-fold higher than WEHI 231 (see Ref. 6 and data not shown). These results indicate that the endogenous enhancer assumes a more extended chromatin configuration as a direct or indirect consequence of ectopic Bright overexpression.

**DISCUSSION**

Herrscher et al. (13) described Bright as a B cell-specific transcription factor capable of transactivating expression from the IgH enhancer ($E_\mu$). In this report we have characterized the contextual requirements of Bright transactivation to further understand how it, and potentially other MAR binding factors, can affect transcription levels. The data presented in this report support several mechanisms for Bright-mediated transcriptional regulation.

Using transient transfection analysis we have demonstrated that context is important for Bright transactivation. Bright was unable to transactivate gene expression from a concatamerized binding site, suggesting that it required interaction with specific factors to function. Furthermore, Bright only acts through the P2 and P4 sites of the $E_\mu$ MARs. This was initially surprising, because Bright binds the P3 site as strongly as P2 and suggested spatial constraints for the interactions of Bright with other factors. This suggested that Bright might function to form tertiary structures of the enhancer DNA and interact with additional DNA-binding proteins or adaptor molecules. In support of this, we demonstrated that Bright distorts DNA. Studies with the T cell receptor $\alpha$-chain enhancer have shown the requirement for DNA bending and distortion to remodel DNA so that transcription factors whose binding sites are spatially distant can interact (46). It is possible that Bright plays a similar role in the induced immunoglobulin expression of late stage B cells.

Synergy between promoter and enhancer transactivation in both the transient and stable transfections suggest an additional level of function for Bright. Because Bright exists in a tetrameric form, and only two functional chains are required for Bright binding in a gel shift assay (13), it is likely that one Bright molecule could bind two sites. Indeed, these studies suggest that Bright could bring an enhancer in apposition to the promoter and directly affect transcriptional activation.
The strategy for inducing MAR binding complexes (reviewed in Ref. 36). We have no evidence for Bright in this remodeling. However, both known classes of bending properties, it is tempting to consider a direct role for Ectopic overexpression of Bright revealed an altered pattern of chromatin organization within the IgH enhancer in WEHI 231 B cell nuclei. Consistent with previous studies (44, 47, 49), the pattern of untransfected WEHI 231 nuclei is restricted to the Eμ core. The assembly of this complex, as judged by in vivo dimethyl sulfate methylation patterns, has been shown to be independent of the flanking MARs (47). Under conditions where Bright is expressed at high levels, DNase I hypersensitivity appears to extend upstream, to include the high affinity P2 site-containing MAR, but not downstream of the core. This third, highly extended configuration extending across the 3' MAR is observed in the two IgM-secreting plasmacytomas that transcribe μ at ~50-fold higher levels. Similarly, the Igκ 3' enhancer assumes three states of DNase I detectable accessibility, which correlate strictly with stage of B cell development (50). That the Bright overexpressing cells may have begun to transition from mature to activated is consistent with the increased Eμ accessibility and the slightly increased levels of μ transcription observed here and with the appearance of active Bright-MAR binding complexes both in normal B cell populations and in B cell lines observed previously (13, 19). Based on its SWI1 similarities, nuclear matrix residence, and MAR binding properties, it is tempting to consider a direct role for Bright in this remodeling. However, both known classes of chromatin remodeling enzymes, SWI1-SNF and the histone acetyltransferases, exist as large multicomponent, ATP-hydrolyzing complexes (reviewed in Ref. 36). We have no evidence for or against participation of Bright as a B cell-restricted member or recruiter of either. However, MARs do confer local regions of transcription (8, 44, 47). In comparing transgenic μ expression in lines generated from wild-type and MAR-deleted Eμ constructs, no Vμ-initiated transcripts were detected from the MAR-deleted locus (47). Using a different approach, Artandi et al. (48) demonstrated that TFE3 proteins binding in the Ig promoter and enhancer could cooperate when binding sites were placed proximal and distal of a CAT gene, presumably through interaction of two dimers. Bright already exists as a tetramer and so would not require any additional protein-protein interactions to carry out this function.

This study also provides functional evidence for the MAR binding function of Bright. Transient transfections with the S107 promoter fragment, which contains a MAR (45), demonstrated that Bright was unable to transactivate from this site. In contrast, when this construct was stably transfected, Bright was now able to affect a 4-fold increase in transcription consistent with the concept that MARs only have effects when they are integrated into the chromosome. We previously demonstrated that Bright protein can be matrix-associated (13). The fact that Bright is only capable of transactivating from the S107 promoter only when it is integrated suggests that Bright can function by modifying or mediating matrix attachment. One difference between the S107 plasmid and the construct with Eμ in the proximal position, which can mediate transactivation in a transient assay, may be the availability of other interacting co-factors. This highlights the context-dependent activity of Bright. Bright may interact with some factors during a transient assay and allow activation from Eμ, whereas matrix attachment is required for transactivation from a substrate that may have limited DNA binding factors associated with it for Bright interaction. In support of interactions such as this, we have recently shown that Bright associates with members of the Sp100 family, which co-localize with Bright in nuclear domains and act as co-factors in transactivation (20). Thus, Bright has multiple requirements for transactivation activity, but the context-dependent activity may also provide multiple mechanisms for Bright to activate gene transcription.

This effect would be consistent with studies that have implicated the IgH enhancer MARs in long range (Ig heavy chain variable gene segment promoter-mediated) transcriptional activation (8, 44, 47).
histone acetylation (51). In a different target gene system, Cux has been shown to form a complex with histone deacetylase that leads to gene inactivation (52). Bright could mediate de-repressive chromatin remodeling indirectly through its successful competition with Cux/histone deacetylase. In a similar logic, Bright, along with related chromatin remodeling proteins, would then be in a position to clear out regions carrying the cis-acting regulatory elements of the core, contributing to the accessibility of conventional DNA binding transactivators to promoter and enhancer elements.

Studies presented in this report suggest some novel mechanisms for the regulation of immunoglobulin gene expression. They confirm that Bright acts in a restricted manner by binding specific sites in the IgH promoter and enhancer and by potentially interacting with other factors within the enhancer core. It further provides some insight into the mechanism of enhancer function and more specifically, how Bright may play an important role in Ig gene expression. Further analysis of these Bright new alternatives should yield a greater understanding of long-standing questions regarding gene regulation.

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