Virology 405 (2010) 524–529

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

FR-like EBNA1 binding repeats in the human genome

Aymeric Fouquier d'Hérouël [a,*], Anna Birgersdotter [b], Maria Werner [a]

[a] Department of Computational Biology, Royal Institute of Technology, AlbaNova University Center, SE-10691 Stockholm, Sweden
[b] Department of Medicine, Division of Hematology, CMM, L8;00, Karolinska Institute, SE-17176 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 28 January 2010
Returned to author for revision
15 February 2010
Accepted 22 June 2010
Available online 22 July 2010

Keyword:
Epstein–Barr virus
EBNA1
Family of Repeats
Human binding sites

ABSTRACT

Epstein–Barr virus (EBV) is widely spread in the human population. EBV nuclear antigen 1 (EBNA1) is a transcription factor that activates viral genes and is necessary for viral replication and partitioning, which binds the EBV genome cooperatively. We identify similar EBNA1 repeat binding sites in the human genome using a nearest-neighbor positional weight matrix. Previously experimentally verified EBNA1 sites in the human genome are successfully recovered by our approach. Most importantly, 40 novel regions are identified in the human genome, constituted of tandemly repeated binding sites for EBNA1. Genes located in the vicinity of these regions are presented as possible targets for EBNA1-mediated regulation. Among these, four are discussed in more detail: IQCB1, IMPG1, IRF2BP2 and TPO. Incorporating the cooperative actions of EBNA1 is essential when identifying regulatory regions in the human genome and we believe the findings presented here are highly valuable for the understanding of EBV-induced phenotypic changes.

Introduction

The Epstein–Barr virus (EBV) is a human herpes virus that infects the majority of the human population. The virus is shed into and spread via saliva. Primary infection is believed to occur in the epithelial cells of the oropharynx, but in order to establish a persistent latent infection the virus targets the B-lymphocytes (Borza and Hutt-Fletcher, 2002; Petgel et al., 2004). The host–virus interaction can be divided into four different latency phenotypes, defined by which viral proteins are expressed, which viral promoters are active and the differentiation stage and type of the infected cell (Babcock et al., 2000). In the latently infected B-lymphocyte, EBV maintains its 170 kbp genome as multiple episomes. One protein is expressed in all infected phenotypes: the Epstein–Barr nuclear antigen 1 (EBNA1). As EBNA1 is involved in viral transcriptional activation, replication and plasmid partitioning, it is indispensable for the virus. The EBV genome is copied once in every S-phase by the host cell replication machinery. This is controlled by *oriP*, a 1.7 kb region of the EBV chromosome that supports the replication and stable maintenance of the EBV plasmids in human cells. It contains two essential components called the Dyad Symmetry (DS) and the Family of Repeats (FR), respectively, both of which contain multiple binding sites for EBNA1 (Resiman and Sugden, 1986; Summers et al., 1996). EBNA1 activates *oriP* by binding to these two regions. The DS region contains four overlapping EBNA1 sites and is the site for initiation of replication. FR is a region with 30 bp repeats containing 20 binding sites for EBNA1, and it acts as a transcriptional enhancer on the viral C promoter, controlling production of all viral nuclear antigens (Resiman and Sugden, 1986). Between six and eight EBNA1 proteins are required in order to fully activate the C promoter (Wysokenski and Yates, 1989; Zetterberg et al., 2004). FR also contain binding sites for the human transcription factor Oct-2 (Almqvist et al., 2005). Multiple EBNA1 homo-dimers are thus required for the formation of a transcriptionally active Cp complex; a process that involves competition with Oct-2 for binding at FR as well as EBNA1-induced changes in the chromatin structure, including DNA looping and nucleosome destabilization (Zetterberg et al., 2004; Frappier and O'Donnell, 1991; Su et al., 1991; Avolio-Hunter et al., 2001; Werner et al., 2007a,b). EBNA1 binding at FR also helps to activate replication from DS through a cooperative action (Wysokenski and Yates, 1989). In addition to FR and DS, EBNA1 also binds to two sites downstream of the viral Q promoter and to two sites in the *oriP* called Rep* (Sample et al., 1992; Wang et al., 2006).

The binding of EBNA1 to DNA is sequence specific, and the requirements for EBNA1 binding are fairly well understood. The crystal structure of DNA-bound EBNA1 has identified the protein domains responsible for DNA recognition and biochemical studies have focused on the exact composition of the DNA binding site and how single nucleotide mutations affect the binding affinity [Ambinder et al., 1990, 1991; Bochkarev et al., 1996]. The binding of EBNA1 to its four different locations on the EBV genome differs dramatically regarding binding strength, with the strongest binding to FR followed by DS, Rep* and Qp (Ambinder et al., 1990; Wang et al., 2006).

Due to its various roles, many attempts have been made to understand how EBNA1 affects human cells and whether EBNA1 can in fact be categorized as an oncogene (Schulz and Cordes, 2009). EBNA1 transgenic mice have been shown to develop malignancies,

* Corresponding author.
E-mail addresses: afd@kth.se (A.F. d'Hérouël), anna.birgersdotter@ki.se (A. Birgersdotter), mariawer@kth.se (M. Werner).

0042-6822/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2010.06.040

and the rate of lymphoma development has been linked to EBNA1 mRNA level (Wilson and Levine, 1992; Wilson et al., 1996). EBNA1 also affects cell proliferation in Burkitt's lymphoma (BL) cell lines, which is partly explained by its interaction with HAUSP/USP7, a deubiqitination enzyme that prevents p53 degradation (Kennedy et al., 2003; Sengupta et al., 2006; Holowaty et al., 2003). Moreover, several studies have shown that EBNA1 can affect the expression of specific genes. Expression of EBNA1 in Hodgkin lymphoma cell lines increased the expression of CD25 and CCL20 (Kube et al., 1999; Baumforth et al., 2008). In Ad/AH cells, EBNA1 can modulate the TGFβ pathway by increasing the degradation of SMAD2 (Flavell et al., 2008). Furthermore, EBNA1 expression in Ad/AH cells and NPC tumors leads to higher expression of certain cytokines and angiogenic factors through increasing AP-1 activity. This is due to increased expression of subunits of the AP-1 dimers: ATF2 and c-Jun. EBNA1 has been shown to bind to their promoters (O'Neil et al., 2008). In addition, EBNA1 increases expression of the V(D)J recombinases RAG1 and RAG2 (Srinivas and Sixbey, 1995). These findings suggest that EBNA1 might facilitate recombination events and could thereby contribute to the c-myc (Ig) locus translocation that is crucial for the development of BL. A recent study shows that EBNA1 induces chromosomal abnormalities by activating transcription of NADPH oxidase, NOX2, thereby increasing the production of reactive oxygen species (ROS) (Gruhne et al., 2009). The authors propose that EBNA1 binds to a site upstream of the NOX2 promoter, since this site resembles binding sites in DS and Qp (Gruhne et al., 2009). In a recent study by Dresang et al. (2009), EBNA1 binding sites were sought for in 27,000 human promoter regions by computational means, followed by experimental verification. This first large scale attempt to scan for EBNA1 sites on the human genome reports 73 in-vitro binding sites. However, the functionality of the reported EBNA1 binding sites remains uncertain, as no significant changes in gene expression could be attributed to the occupation of these sites (Dresang et al., 2009). We believe that since the transcriptional activation process with EBNA1 on the EBV genome is known to be highly cooperative, it is likely that EBNA1 also binds to multiple sites on the human genome, when functioning as an activator or repressor of human genes. Hence, the aim of this study is to investigate whether it is possible to identify FR-like regions in the human genome. From a bioinformatical screening of the whole human genome, we filtered out 40 potential FR-like regions with fairly strong predicted binding affinity for EBNA1. Several of them are located in the vicinity of protein-coding genes that can be related to EBV infections.

Results

Binding sites on the EBV genome

First the matrix was evaluated on the EBV genome. Each position on the genome is assigned a score which is used as a proxy for the binding affinity for EBNA1. The lower a site's score, the higher its similarity to the original set of binding sites, thus a lower score correlates to an increased likelihood of EBNA1 binding. The background, that is the score given by EBNA1 binding to sites that are not specific binding sites, includes in this study scores down to −8, i.e. the same order as the binding sites of Rep*and Qp. Our matrix correctly identifies all previously known sites. The obtained scores for the EBNA1 binding sites on the EBV genome range from −14.3 to −19 for FR, see inlay in Fig. 1, and from −10.8 to −15.1 for the DS sites. The weaker scores for Qp (−8.7 and −12.7) and Rep* (−7 and −8.9) led us to define the threshold of −10 for the filtering of the human genome scores. Using a single nucleotide weight matrix description instead of the nearest neighbor model, leads to a much smaller score gap between known binding sites and non-specific background. The difference between both descriptions is apparent in Fig. 1.

Single EBNA1 binding sites on the human genome

Before looking at the repeated regions that bind EBNA1, we examined the amount of predicted binding sites for EBNA1 on the human genome. A threshold of −8, set as a first cutoff, yielded $2.3 \cdot 10^6$ predicted binding sites on the human genome. Fig. 2 shows the distribution of these scores. As FR-like regions are the focus of this study, these hits have not been the subject of further investigation.

Repeated binding sites

EBNA1 activation of the C and W promoter in the EBV genome is a highly cooperative process involving the occupation of multiple binding sites (Wysokenski and Yates, 1989; Zetterberg et al., 2004; Werner et al., 2007b). Based on this, we focused on the identification of human FR-like regions. After the refined filtering (see Methods), we retained a total of 50 FR-like regions on the whole genome for which the nearest protein-coding genes were determined. The distribution of these regions throughout the human chromosomes is shown in Fig. 3.

Ten of these regions were neighboring uncharacterized genes and therefore excluded from further analysis. Table 1 lists the remaining 40 genes.

EBNA1 binding scores at already predicted sites

Gruhne et al. (2009) recently showed that EBNA1 induces chromosomal aberrations, DNA double-strand breaks and engages the DNA damage response. According to their finding, NOX2 oxidase is transcriptionally active in EBNA1 expressing cells, and there is a possible binding site for EBNA1 in the promoter of NOX2. This site is similar to the Qp and DS site, i.e. it is most likely a weak binding site for EBNA1. Indeed, in our screening we do not recognize this site, and when evaluating our matrix it yields a score of −5.16 which indicates that the site is highly different from FR and DS and might have an extremely low binding strength, weaker than Qp and Rep*, and alternatively needs secondary factors to ensure binding.

O'Neil et al. describe c-Jun and ATF2 as likely regulatory targets of EBNA1. They were not able to show direct binding of EBNA1 to these promoter-regions. However, they argue for a likely involvement of EBNA1 in direct regulation of these two genes. We looked in our predicted binding site library for sites with a better score than −10, in the surroundings of these two transcription start sites. For c-Jun, we identify one EBNA1 site of score −12.3, 2298 bp upstream of the transcription start site (NCBI36.48.dna.chromosome.1: 59024885). For ATF2, there is one site with a score of −10.5, 4263 bp upstream and one site of with a score of −11.55, 2908 bp downstream of the transcription start site (NCBI36.48.dna.chromosome.2: 175745443 and 175734088). Since RAG1 and RAG2 were seen to be up-regulated in the presence of EBNA1 in the cell, their promoter sequences were also queried for EBNA1 binding sites. However, our matrix does not identify any likely binding sites for EBNA1 in the vicinity of these two promoters.

We further verified whether our method correctly identifies the EBNA1 binding sites found by Dresang et al. Our method recovers 53 out of the 73 previously experimentally identified EBNA1 binding sites with scores above background (Supplementary Table 1).

Discussion

The aim of this study was to identify FR-like regions in the human genome; more precisely, regions with multiple binding sites for EBNA1, with fairly high predicted binding scores and non-overlapping with mobile DNA elements. After the stepwise filtering we were left with 40 regions. These are presented in Table 1 and we believe that a number of these proteins are interesting in relation to EBV.

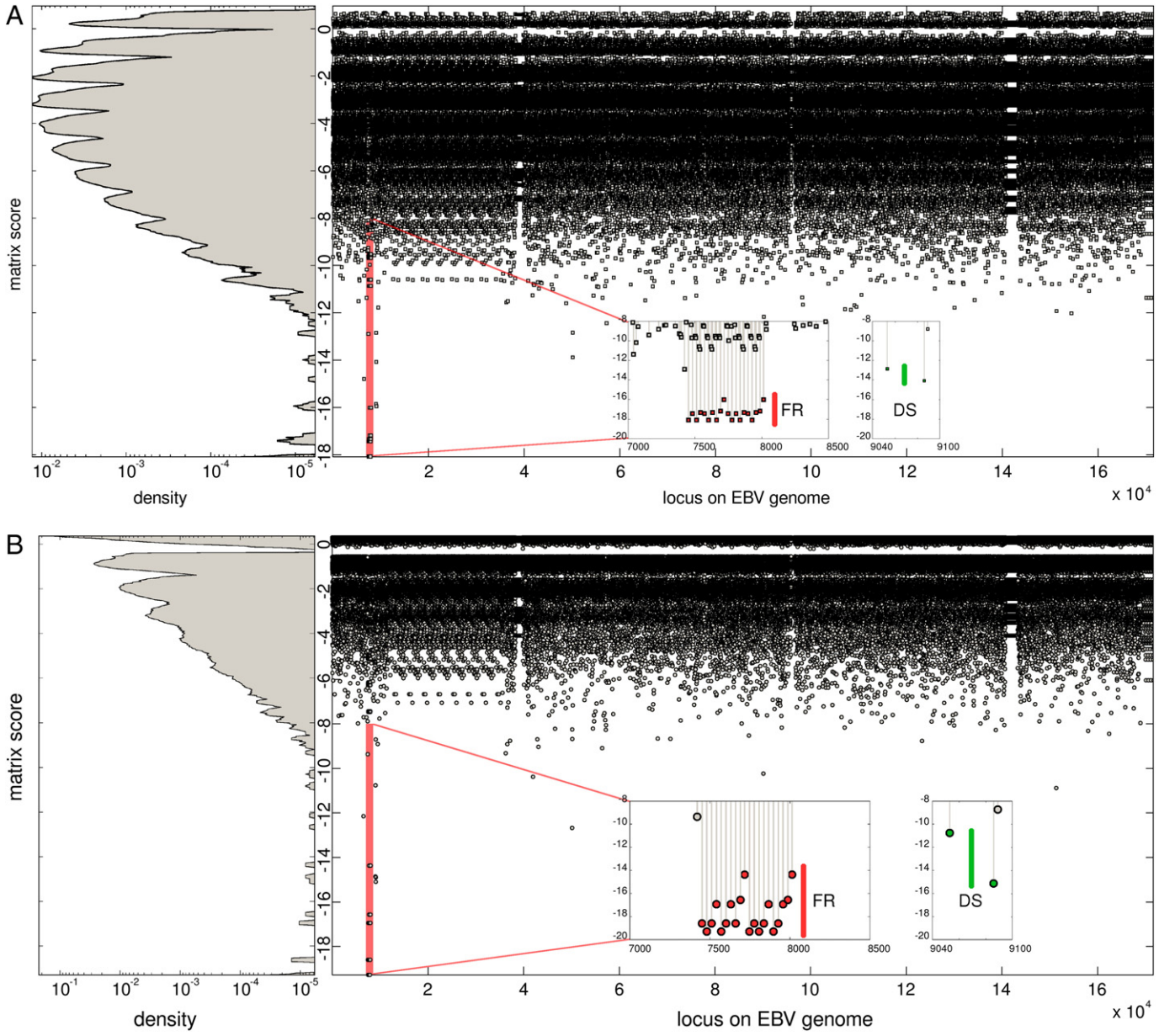

Fig. 1. Distribution and positioning of predicted binding sites on EBV. A) Figure of the score distribution and the positioning of the predicted EBNA1 binding sites on the EBV genome, using a single nucleotide weight matrix. The background level is quite high, around −10, as can be seen both on the EBV map (right plot) as well as on the score density function (left plot). The sites of FR are however still distinguishable (left inlay). B) The same plots of score distribution and the positioning of the predicted EBNA1 binding sites on the EBV genome; this time using our nearest neighbor weight matrix. The background level is significantly lower using this model, lowering the risk for false positive sites with a threshold of −10 as base for FR-like regions. The left inlay shows the FR region of EBV, now significantly more prominent, while the DS region is shown in the right inlay.

The two genes with the largest negative scores, IQ-motif Ca2+ binding protein 1 (IQCB1) and interphotoreceptor matrix proteoglycan 1 (IMPG1) are, surprisingly, both associated with photoreceptors. IQCB1 is expressed and localized in the primary cilia of renal epithelial cells, but it also functions as a bridge between p53 and calmodulin-regulated cellular processes in epithelial cells (Luo et al., 2005a). Functionally IQCB1 binds to both the Ca2+-bound and Ca2+-free calmodulin, and its expression is down-regulated by p53 and genotoxic agents. Thus by negatively regulating IQCB1, p53 may further enhance PUMA (p53 up-regulated modulator of apoptosis) expression. IQCB1 is up-regulated in a number of primary colorectal and gastric tumors when compared with matching normal tissues (Luo et al., 2005a). Transcriptional up-regulation of PUMA modulates endoplasmic reticulum calcium pool depletion-induced apoptosis via Bax activation (Luo et al., 2005b). IQCB1 is expressed by the EBV positive BL cell line Daudi (http://biogps.gnf.org/). Hypothetically, this gene could add a survival advantage if expressed at a higher level and at specific time points during division and differentiation. It would be interesting to further explore if EBNA1 can up-regulate this gene and whether this could be related to EBNA1 directed tumorigenicity. IMPG1/SPACR (sialoprotein associated with cones and rods), is the major 147–150 kDa glycoprotein present in the insoluble interphotoreceptor matrix of the human retina (Acharaya et al., 1998). Little is known about this protein but it is expressed in EBV positive BL cell lines Daudi and Raji (http://biogps.gnf.org/) and it would be worth investigating whether IMPG1, like IQBC1, can play various roles in different cellular backgrounds.

One of the identified FR-like regions is located close to the gene encoding IRF2BP2, the Interferon Regulatory Factor-2 binding protein 2. IRF2BP2 is an IRF2-dependent co-repressor, interacting with the C-terminal of the IRF2 protein (Childs and Goodbourn, 2003). IRF2 is both a transcriptional activator and repressor, involved in regulation

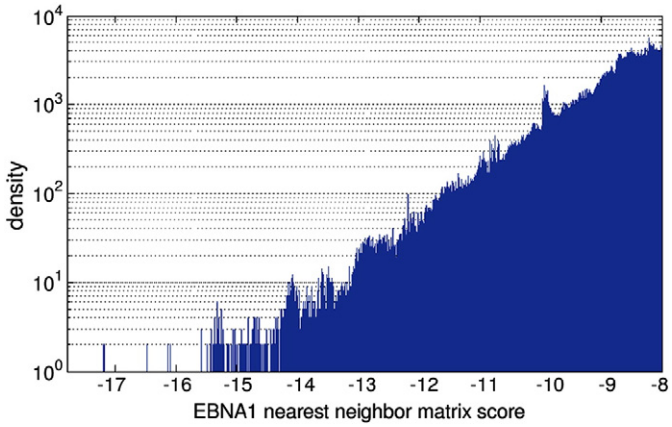

Fig. 2. Score density function of single EBNA1 sites on the human genome. Given that some of the known functional EBNA1 sites on EBV have a binding site score of the order of −8, using our model, one should pay attention to the large number of predicted single sites within this order of score. It gives an indication on the predicaments of looking at single EBNA1 binding sites on the human genome. However, there is also a number of sites with predicted binding affinity of the same order as FR.

of interferon genes as response to viral infections (Harada et al., 1989). What is highly interesting with the finding of an eventual EBNA1 regulative motif close to IRF2BP2 is that IRF2 has been shown to activate EBNA1 transcription from the viral Q promoter (Schaefer et al., 1997; Nonkwelo et al., 1997). It is difficult to hypothesize around the exact regulatory role of our FR-like region, but there might indeed be a feedback mechanism involved here, where EBNA1 indirectly affects the IRF2 transcriptional activation abilities; on Qp as well as on other promoters.

Another interesting protein in our list of genes is the thyroid peroxidase (TPO). TPO is targeted by autoantibodies produced in Hashimoto's thyroiditis, an autoimmune thyroid disease (Chardes et al., 2002). Hashimoto's disease is a form of hypothyroiditis which has been associated with EBV infections in several studies (Vrbikova et al., 1996; Desailloud and Hober, 2009; Shimon et al., 2003). However, whether the virus is an innocent bystander or actively plays a role in inducing the disease is still not understood. It would be of high interest to further investigate the functionality of the eventual binding region we identified close to the thyroid peroxidase gene, measuring the effect of EBNA1 binding on TPO expression. This would give a clearer insight into a possible correlation between the EBV infection and autoimmune thyroid disorders.

EBNA1 has been shown to increase the migration potential of the infected cell by binding to the metastasis inhibitor NM23-H1, thereby changing its localization from the cytoplasm to the nucleus. This relocalization inhibits NM23-H1 non-metastatic effect (Murakami et al., 2005). EBNA1 might have other ways to affect the migration potential of the cell, as FR-like repeats are found in the enhancer regions of Profilin and Tropomyosin (PFN2 and TMP1). Profilin is an actin-binding protein involved in the spatially and temporally controlled growth of actin microfilaments, which is an essential process in cellular locomotion and cell shape changes. The TMP1 gene codes for four different Tropomyosin isoforms. Tropomyosin is also an actin-binding protein and is involved in actin remodeling (Lindberg et al., 2008).

It has further been shown that EBNA1 decreases the half-life of SMAD2 and thereby modulates TGFβ signaling. SMURF2 targets SMAD2 for degradation, as do WWP1 and NEDD4-2 (Flavell et al., 2008). Our results show that EBNA1 binds enhancer regions to several genes involved in protein degradation, thus it would be interesting to further analyze their targets and verify if SMAD2 is among those, especially as the protein that targets nuclear SMAD2 for degradation is unknown (Zhang et al., 2001).

The experimental search for EBNA1 binding sites by Dresang et al. was limited to promoter regions of the EBV-positive lymphoblastoid cell

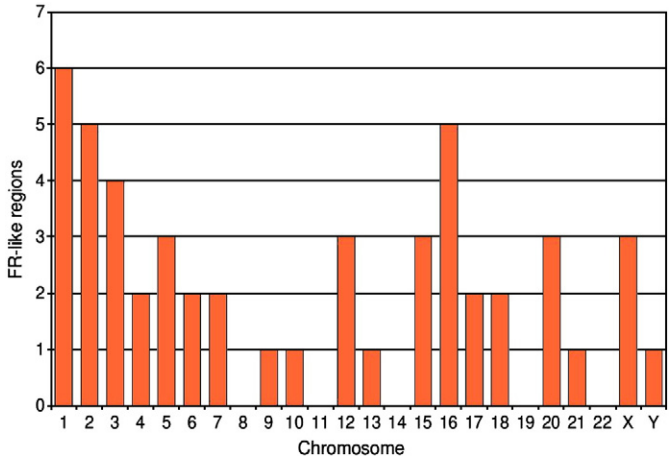

Fig. 3. Number of identified FR-like regions per chromosome on the human genome.

Table 1
List of 40 FR-like regions identified in this study.

Chr[a]	Best score[b]	Spacing[c]	Nearest gene[d]	Distance[e,g]	Sites[f]
1	−10.33	21	AGL	−48,442	3
1	−11.46	25	IRF2BP2	157,348	3
1	−10.36	35	KCNK2	10,828	3
1	−12.37	30–37	USP24	601,282	7
2	−11.61	28	HAT1	8667	13
2	−10.18	20	SLC4A3	−718,192	3
2	−12.62	29–31	SPTBN1	50,317	4
2	−11.27	29–30	TGFA	−54,992	6
2	−10.99	33	TPO	5235	3
3	−15.29	18	IQCB1	−337	5
3	−10.42	37	PFN2	116,075	3
3	−14.16	18	ROBO2	−461,277	5
3	−11.85	17	TBL1XR1	−85,471	3
4	−11.85	36	NR3C2	939,768	4
4	−10.77	34	SNX25	−11,808	6
5	−11.61	24	CCDC99	293,716	4
5	−11.61	17	EDIL3	723,310	3
5	−12.01	22	SEMA5A	−493,777	3
6	−15.26	23–24	IMPG1	263,802	5
6	−12.05	39	PSMB9	−1450	3
7	−11.74	25	HUS1	−24,381	4
7	−10.25	42	MPP6	23,033	3
10	−10.46	44	INPP5A	−94,516	3
12	−10.40	29–32	DTX1	18,763	4
12	−11.16	25	TBX3	612,914	4
13	−12.20	22	MYCBP2	4164	3
15	−11.34	37	ABHD2	−12,612	3
15	−11.01	25–30	TPM1	98,263	6
16	−11.43	28	SOX8	−31,090	5
16	−10.22	44	SOX8	−27,876	4
17	−10.04	36	ALOXE3	10,674	3
17	−11.92	23	NLRP1	33,063	7
18	−11.08	16	CDH20	459,550	3
18	−10.90	16–20	RIOK3	18,869	4
20	−12.74	20	C20orf100	26,514	4
20	−10.20	27	PLCB1	−104,278	3
21	−10.24	19	IGHV1OR15–9	2,690,575	3
X	−10.74	38	AMELX	−185,093	3
X	−10.24	22–36	ZCCHC13	12,894	7
Y	−10.82	33	USP9Y	107,897	3

[a] Chromosome.
[b] Best score among sites in region.
[c] End-to-end spacing between neighboring sites.
[d] Symbol of nearest protein-coding gene.
[e] Distance to nearest protein-coding gene transcription start.
[f] Number of binding sites in region.
[g] Negative distances correspond to regions upstream of the transcription start site of the gene.

line 721, probed by NimbleGen's ChIP-chip human promoter arrays. Our method remains unbiased, both towards cell-type specific chromatin structure or nucleosome positioning, and *a priori* restrictions on the queried sequences. The latter is of special interest, since eukaryotic regulatory elements comprise not only promoter-near transcription factor binding sites, but also enhancer-, silencer- and insulator sequences, which can be located several kb up- or downstream of a corresponding promoter (Narlikar and Ovcharenko, 2009; Blackwood and Kadonaga, 1998). A recent study identifies clusters of EBNA1 binding sites in human promoter regions (Canaan et al., 2009). This finding underlines the value of focusing on multiple binding sites when searching for functional EBNA1 sites.

Positional weight matrices assuming single nucleotide models are widely used both for representation of DNA binding sites, e.g. as WebLogos (Crooks et al., 2004), and for bioinformatical identification of putative binding sites. However, more subtle statistical features than average positional nucleotide occurrences are not reflected by such objects. Weight matrices featuring higher order statistics can, on the other hand, always be reduced to a lower order representation. DNA binding sites are known to be more subtly recognized by proteins than by the recognition of isolated nucleotides (for a more thorough discussion see e.g. (O'Flanagan et al., 2005)). We therefore advocate for a broader use of higher order (at least nearest neighbor) positional weight matrices, as they are able to reflect a more acute description of binding sites.

EBNA1 is known for binding to its EBV sites with extremely varying affinity. From 73 experimentally identified human binding sites for EBNA1, none was successfully shown to be functional (Dresang et al., 2009). The putative EBNA1 site in the NOX2 promoter (Gruhne et al., 2009) scores much weaker than the sites on Qp or Rep* within our model. The data presented in this paper needs to be verified in biological systems and different experimental platforms by comparing transcript and protein levels of target genes in EBNA1 positive and negative cells. It remains intriguing to elucidate whether genes presented in this study are involved in tumor progression, for example IQCB, TMP1 and PFN2. Also EBNA1 binding at the suggested sites must be explored more in depth. Altogether, the difficulty of correctly identifying isolated binding sites for EBNA1 becomes evident. This may be due to the manifold roles of EBNA1 as a regulatory protein rendering a unique description in terms of a single binding motif impossible. To exert its function, EBNA1 might rely on higher orders of control, either by cooperating with other transcription factors or by binding multiple sites, possibly in competition with other factors. This was our motivation for searching specifically for tandemly repeated EBNA1 sites. We believe that the cooperative nature of EBNA1 is an important factor when searching for regulatory binding sites of this protein on the human genome.

Methods

Nearest neighbor position weight matrix

Weight matrices were constructed from the binding sites of FR on EBV (AJ507799). Di-nucleotide frequencies $f_{\eta\nu}$ were calculated from the 20 binding sites of FR and human chromosomal di-nucleotide occurrence probabilities $p^c_{\eta\nu}$ were taken into account to construct the matrices according to

$$w^c_{\eta\nu,i} = -f_{\eta\nu,i}\log\frac{f_{\eta\nu,i}}{p^c_{\eta\nu}}.$$

A given sequence $\mathrm{s} = (s_1 s_2 \ldots s_n)$ has then the associated score

$$I(\mathrm{s}) = \sum_{k=1}^{n-1} w^c_{s_k s_{k+1},k},$$

which is the more negative the more S resembles a sequence from FR and the more it differs from background sequences modeled by $p^c_{\eta\nu}$. Evaluating the matrix on a long sequence $\mathbf{S}$ ($|\mathbf{S}| > n$), e.g. a whole chromosome, then means calculating $I(\mathrm{s})$ of each sub-sequence $\mathrm{s}\subset\mathbf{S}$.

Screening of EBV and the human genome

The EBNA1 matrix was applied to the EBV genome (AJ507799) and the complete human genome sequence (NCBI36 Ensembl release 48). Since FR-like sequences may be masked by common repeat recognition software, the unmasked genome was used for our analysis. Sites with weaker scores than −8 were discarded as background. The remaining sites were stepwise filtered to identify FR-like regions. Regions containing a minimum of three consecutive sites of which at least one of the sites scored better than −10 were kept as potential FR-like regions. Allowed end-to-end distance between sites were 16 to 50 nucleotides. Regions overlapping with annotated (RepeatMasker) mobile DNA elements were excluded. Functional binding site repeats are assumed to be under constant selection pressure and may exhibit small sequence variations between individual sites, as is the case for FR and DS on EBV. Therefore, as a final filter we excluded perfect tandem repeats that did not exhibit any variations.

Acknowledgments

This work was supported by the Swedish Science Council (M.W. and A.F.d'H.) and the Stockholm County Council (A.B.). The authors would like to thank Ingemar Ernberg for challenging and fruitful discussions concerning EBV and Erik Aurell for useful comments on the manuscript. A.B. would like to thank Magnus Björkholm and Jan Sjöberg for financial and scientific support and Erik Aurell for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.virol.2010.06.040.

References

Acharaya, S., Rayborn, M.E., Hollyfield, J., 1998. Characterization of SPACR, a sialoprotein associated with cones and rods present in the interphotoreceptor matrix of the human retina: immunological and lectin binding analysis. Glycobiology 8, 997–1006.

Almqvist, J., Zou, J., Linderson, Y., Borestrom, C., Altiok, E., Zetterberg, H., Rymo, L., Pettersson, S., Ernberg, I., 2005. Functional interaction of Oct transcription factors with the family of repeats in Epstein–Barr virus oriP. J. Gen. Virol. 86, 1261–1267.

Ambinder, R.F., Mullen, M.A., Chang, Y.N., Hayward, G.S., Hayward, S.D., 1991. Functional domains of Epstein–Barr virus nuclear antigen ebna-1. J. Virol. 65 (3), 1466–1478.

Ambinder, R.F., Shah, W.A., Rawlins, D.R., Hayward, G.S., Hayward, S.D., 1990. Definition of the sequence requirements for binding of the ebna-1 protein to its palindromic target sites in Epstein–Barr virus DNA. J. Virol. 64 (5), 2369–2379.

Avolio-Hunter, T.M., Lewis, P.N., Frappier, L., 2001. Epstein–Barr nuclear antigen 1 binds and destabilizes nucleosomes at the viral origin of latent DNA replication. Nucleic Acids Res. 29 (17), 3520–3528.

Babcock, G.J., Hochberg, D., Thorley-Lawson, D.A., 2000. The expression pattern of Epstein–Barr virus latent genes in vivo is dependent upon the differentiation stage of the infected B cell. Immunity 13, 497–506.

Baumforth, K.R.N., Birgersdotter, A., Reynolds, G.M., Wei, W., Kapatai, G., Flavell, J.R., Kalk, E., Piper, K., Lee, S., Machado, L., Hadley, K., Sundblad, A., Sjoberg, J., Bjorkholm, M., Porwit, A.A., Yap, L.-F., Teo, S., Grundy, R.G., Young, L.S., Ernberg, I., Woodman, C.B.J., Murray, P.G., 2008. Expression of the Epstein–Barr virus-encoded Epstein–Barr virus nuclear antigen 1 in Hodgkin's lymphoma cells mediates up-regulation of ccl20 and the migration of regulatory T cells. Am. J. Pathol. 173 (1), 195–204.

Blackwood, E.M., Kadonaga, J.T., 1998. Going the distance: a current view of enhancer action. Science 281, 60–63.

Bochkarev, A., Barwell, J.A., Pfuetzner, R.A., Bochkareva, E., Frappier, L., Edwards, A.M., 1996. Crystal structure of the DNA-binding domain of the Epstein–Barr virus origin-binding protein, ebna1, bound to DNA. Cell 84 (5), 791–800.

Borza, C.M., Hutt-Fletcher, L.M., 2002. Alternative replication in b cells and epithelial cells switches tropism of Epstein–Barr virus. Nat. Med. 8, 594–599.

Canaan, A., Haviv, I., Urban, A.E., Schulz, V.P., Hartman, S., Zhang, Z., Palejev, D., Deisseroth, A.B., Lacy, J., Snyder, M., Gerstein, M., Weissman, S.M., 2009. Ebna1

regulates cellular gene expression by binding cellular promoters. Proc. Natl. Acad. Sci. 106 (52), 22421–22426.
Chardes, T., Chapal, N., Bresson, D., Bes, C., Giuducelli, V., Lefranc, M., peraldi Roux, S., 2002. The human anti-thyroid peroxidase autoantibody repertoire in Grave's and Hashimoto's autoimmune thyroid diseases. Immunogenetics 54, 141–157.
Childs, K.S., Goodbourn, S., 2003. Identification of novel co-repressor molecules for interferon regulatory factor-2. Nucleic Acids Res. 31 (12).
Crooks, G.E., Hon, G., Chandonia, J.M., Brenner, S., 2004. Weblogo: a sequence logo generator. Genome Res. 14, 1188–1190.
Desailloud, R., Hober, D., 2009. Viruses and thyroiditid: an update. Virol. J. 6 (5).
Dresang, L.R., Vereide, D.T., Sugden, B., 2009. Identifying sites bound by Epstein–Barr virus nuclear antigen 1 (ebna1) in the human genome: defining a position-weighted matrix to predict sites bound by ebna1 in viral genomes. J. Virol. 83 (7), 2930–2940.
Flavell, J.R., Baumforth, K.R.N., Wood, V.H.J., Davies, G.L., Wei, W., Reynolds, G.M., Morgan, S., Boyce, A., Kelly, G.L., Young, L.S., Murray, P.G., 2008. Down-regulation of the tgf/beta target gene, ptprk, by the Epstein/Barr virus encoded ebna1 contributes to the growth and survival of Hodgkin lymphoma cells. Blood 111, 292–301.
Frappier, L., O'Donnell, M., 1991. Epstein–Barr nuclear antigen 1 mediates a DNA loop within the latent replication origin of Epstein–Barr virus. Proc. Natl Acad. Sci. USA 88 (23), 10875–10879.
Gruhne, B., Sompallae, R., Marescotti, D., Kamranvar, S.A., Gastaldello, S., Masucci, M.G., 2009. The Epstein–Barr virus nuclear antigen-1 promotes genomic instability via induction of reactive oxygen species. Proc. Natl Acad. Sci. USA 106 (7), 2313–2318.
Harada, H., Fujita, T., Miyamoto, M., Kimure, Y., Maruyama, M., Furia, A., Miyata, T., Taniguchi, T., 1989. Structurally similar but functionally distinct factors, IRF/1 and IRF/2, bind to the same regulatory elements of IFN and IFN/inducible genes. Cell 58, 729–739.
Holowaty, M.N., Zeghouf, M., Wu, H., Tellam, J., Athanasopoulos, V., Greenblatt, J., Frappier, L., 2003. Protein profiling with Epstein–Barr nuclear antigen-1 reveals an interaction with the herpesvirus-associated ubiquitin-specific protease HAUSP/USP7. J. Biol. Chem. 278, 29987–29994.
Kennedy, G., Komano, J., Sugden, B., 2003. Epstein–Barr virus provides a survival factor to Burkitt's lymphomas. Proc. Natl Acad. Sci. USA 100 (24), 14269–14274.
Kube, D., Vockerodt, M., Weber, O., Hell, K., Wolf, J., Haier, B., Grsser, F.A., Mller-Lantzsch, N., Kieff, E., Diehl, V., Tesch, H., 1999. Expression of Epstein–Barr virus nuclear antigen 1 is associated with enhanced expression of cd25 in the Hodgkin cell line l428. J. Virol. 73 (2), 1630–1636.
Lindberg, U., Schutt, C.E., Goldman, R.D., Nyakern-Meazza, M., Hillberg, L., Ratjhe, L., Grenklo, S., 2008. Tropomyosins regulate the impact of actin binding proteins on actin filaments. Adv. Exp. Med. Biol. 644, 223–231.
Luo, X., He, Q., Huang, Y., Sheikh, M.S., 2005a. Cloning and characterization of a p53 and DNA damage down-regulated gene PIQ that codes for a novel calmodulin-binding IQ motif protein and is up-regulated in gastrointestinal cancers. Cancer Res. 65, 10725–10733.
Luo, X., He, Q., Huang, Y., Sheikh, M.S., 2005b. Transcriptional upregulation of puma modulates endoplasmic reticulum calcium pool depletion/induced apoptosis via bax activation. Cell Death Differ. 12, 1310–1318.
Murakami, M., Lan, K., Subramanian, C., Robertson, E.S., 2005. Epstein–Barr virus nuclear antigen 1 interacts with nm23-h1 in lymphoblastoid cell lines and inhibits its ability to suppress cell migration. J. Virol. 79, 1559–1568.
Narlikar, L., Ovcharenko, I., 2009. Identifying regulatory elements in eukaryotic genomes. Brief. Funct. Genomic Proteomic 8 (4), 215–230.
Nonkwelo, C., Ruf, I.K., Sample, J., 1997. Interferon-independent and -induced regulation of Epstein–Barr virus ebna/1 gene transcription in Burkitt lymphoma. J. Virol. 71 (9), 6887–6897.
O'Flanagan, R.A., Paillard, G., Lavery, R., Sengupta, A.M., 2005. Non-additivity in protein–DNA binding. Bioinformatics 21 (10), 2254–2263.
O'Neil, J.D., Owen, T.J., Wood, V.H.J., Date, K.L., Valentine, R., Chukwuma, M.B., Arrand, J.R., Dawson, C.W., Young, L.S., 2008. Epstein–Barr virus-encoded EBNA1 modulates the AP-1 transcription factor pathway in nasopharyngeal carcinoma cells and enhances angiogenesis in vitro. J. Gen. Virol. 89 (Pt 11), 2833–2842.
Petgel, D.M., Middeldorp, J., Thorley-Lawson, D.A., 2004. Epstein–Barr virus infection in ex vivo tonsil epithelial cell cultures of asymptomatic carriers. J. Virol. 78 (22), 12613–12624.
Resiman, D., Sugden, B., 1986. *trans* activation of am Epstein–Barr viral transcriptional enhancer by the Epstein–Barr viral nuclear antigen 1. Mol. Cell. Biol. 6 (11), 3838–3846.
Sample, J., Henson, E.D., Sample, C., 1992. The Epstein–Barr virus nuclear protein 1 promoter active in type I latency is autoregulated. J. Virol. 66 (8), 4654–4661.
Schaefer, B.C., Paulson, E., Strominger, J.L., Speck, S.H., 1997. Constitutive activation of Epstein/Barr virus *ebv(nuclear antigen 1 gene transcription by IRF1 and IRF2 during restricted ebv latency. Mol. Cell. Biol. 17 (2), 873–886.
Schulz, T.F., Cordes, S., 2009. Is the Epstein–Barr virus ebna-1 protein an oncogen? Proc. Natl Acad. Sci. USA 106 (7), 2091–2092.
Sengupta, S., den Boon, J.A., Chen, I.-H., Newton, M.A., Dahl, D.B., Chen, M., Cheng, Y.-J., Westra, W.H., Chen, C.-J., Hildesheim, A., Sugden, B., Ahlquist, P., 2006. Genome-wide expression profiling reveals EBV-associated inhibition of MHC class I expression in nasopharyngeal carcinoma. Cancer Res. 66 (16), 7999–8006.
Shimon, I., Pariente, C., Shlomo-David, J., Grossman, Z., Sack, J., 2003. Transient elevation of triiodothyronine caused by triiodothyronine autoantibody associated with acute Epstein–Barr-virus infection. Thyroid 13 (2), 211–215.
Srinivas, S.K., Sixbey, J.W., 1995. Epstein–Barr virus induction of recombinase-activating genes rag1 and rag2. J. Virol. 69 (12), 8155–8158.
Su, W., Middleton, T., Sugden, B., Echols, H., 1991. DNA looping between the origin of replication of Epstein–Barr virus and its enhancer site: stabilization of an origin complex with Epstein–Barr nuclear antigen 1. Proc. Natl Acad. Sci. USA 88 (23), 10870–10874.
Summers, H., Barwell, J.A., Pfuetzner, R.A., Edwards, A.M., Frappier, L., 1996. Cooperative assembly of ebna1 on the Epstein–Barr virus latent origin of replication. J. Virol. 70 (2), 1228–1231.
Vrbikova, J., Janatkova, I., Zamrazil, V., Tomiska, F., Fucikova, T., 1996. Epstein–Barr virus serology in patients with autoimmune thyroiditis. Exp. Clin. Endocrinol. Diab. 104 (1), 89–92.
Wang, J., Lindner, S.E., Leight, E.R., Sugden, B., 2006. Essential elements of a licensed, mammalian plasmid origin of DNA synthesis. Mol. Cell. Biol. 26 (3), 1124–1134.
Werner, M., Ernberg, I., Zou, J., Almqvist, J., Aurell, E., 2007a. Epstein–Barr virus latency switch in human B-cells: a physico-chemical model. BMC Syst. Biol. 1, 40.
Werner, M., Zhu, L., Aurell, E., 2007b. Cooperative action in eukaryotic gene regulation: physical properties of a viral example. Phys. Rev. E Stat. Nonlin. Soft Matter. Phys. 76, 061909.
Wilson, J.B., Bell, J.L., Levine, A.J., 1996. Expression of Epstein–Barr virus nuclear antigen-1 induces B cell neoplasia in transgenic mice. EMBO J. 15 (12), 3117–3126.
Wilson, J.B., Levine, A.J., 1992. The oncogenic potential of Epstein–Barr virus nuclear antigen 1 in transgenic mice. Curr. Top. Microbiol. Immunol. 182, 375–384.
Wysokenski, D.A., Yates, J.L., 1989. Multiple EBNA1-binding sites are required to form an EBNA1-dependent enhancer and to activate a minimal replicative origin within oriP of Epstein–Barr virus. J. Virol. 63 (6), 2657–2666.
Zetterberg, H., Borestrm, C., Nilsson, T., Rymo, L., 2004. Multiple ebna1-binding sites within oripi are required for ebna1-dependent transactivation of the Epstein–Barr virus C promoter. Int. J. Oncol. 25 (3), 693–696.
Zhang, Y., Chang, C., Gehling, D.J., Hemmati-Brivanou, A., Derynck, R., 2001. Regulation of smad degradation and activity by smurf, an e3 ubiquitin ligase. Proc. Natl Acad. Sci. USA 98, 974–979.