Logic based mapping of promoter enhancer regions of HPV16 and Interferon γ - a search for similarity

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The complexity of virus–host interactions arises from the ability of both the host cell and the virus to utilize a variety of strategies to control each other during infection. Cellular transcription factors that are recruited by both the host cells and viruses play a key role in the performance of their functions. Here we compiled the literature on transcription factors that are involved in the regulation of interferon-γ gene expression and in the enhancement of the long control region of the human papillomavirus16, presenting this information in two logic-based diagrams using the System Biology Graphical Notation (SBGN) scheme.

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The Genomics and Pathway Biology MSc programme started in 2003 and has had a number of supervisors and contributors. Profs Peter Ghazal and Douglas Roy have supervised the programme throughout. Other supervisors include Dr Stuart Moodie (2004-5), Dr Kevin Robertson (2004-7), Prof Tom Freeman (2006-7), Dr Steven Watterson (2008-10), Dr Alexander Mazein (2009-11) and Dr Kai Kropp (2010-11).

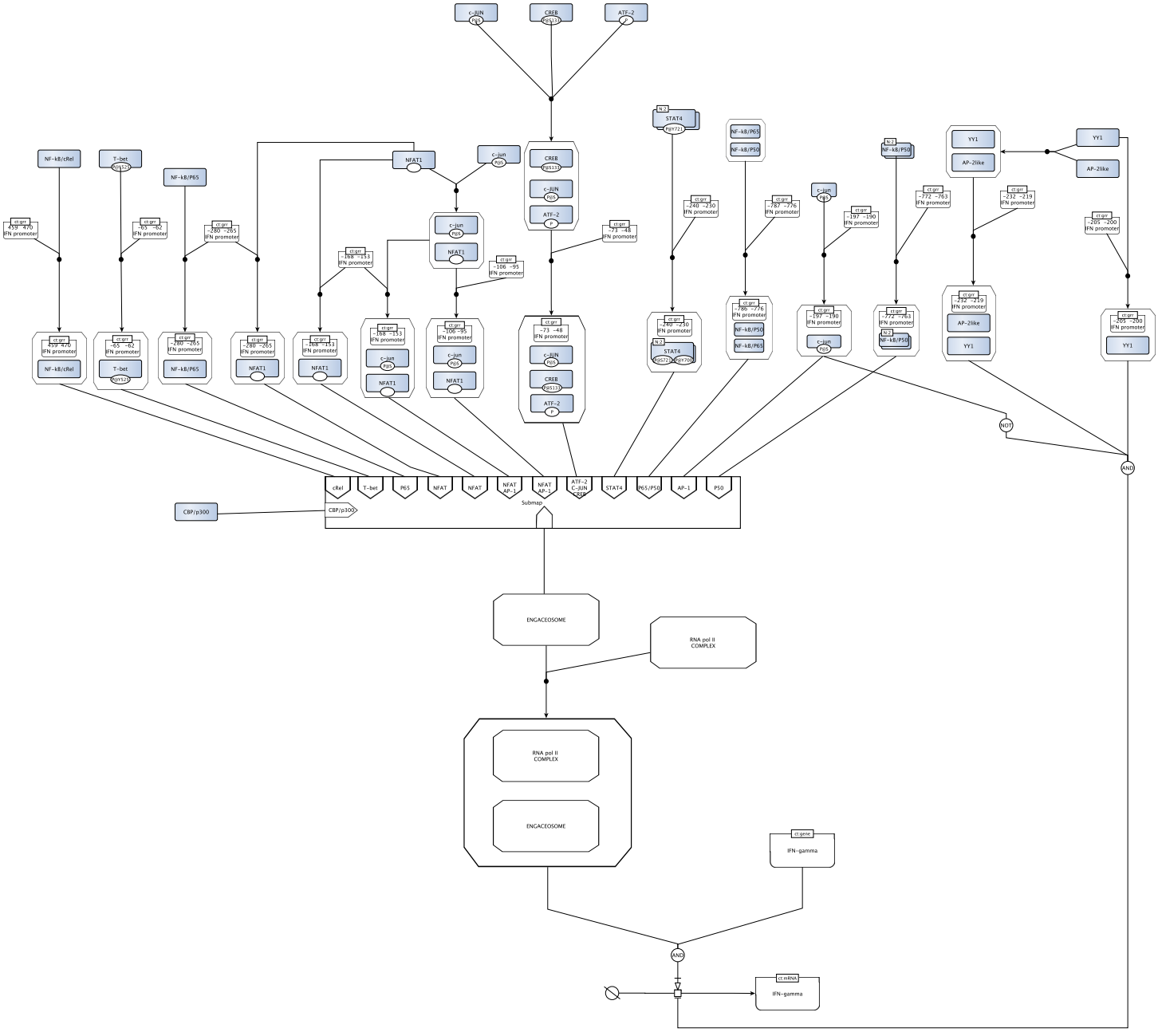

Figure 1: Constructed diagram of the transcription factors that bind IFNγ gene promoter. The SBGN scheme is used to describe the binding sites of the transcription factors and their effects on the regulation of IFNγ production. 14 transcription factors are depicted in their activated phase and their binding sites are within (-790 and -45) of IFNγ promoter.

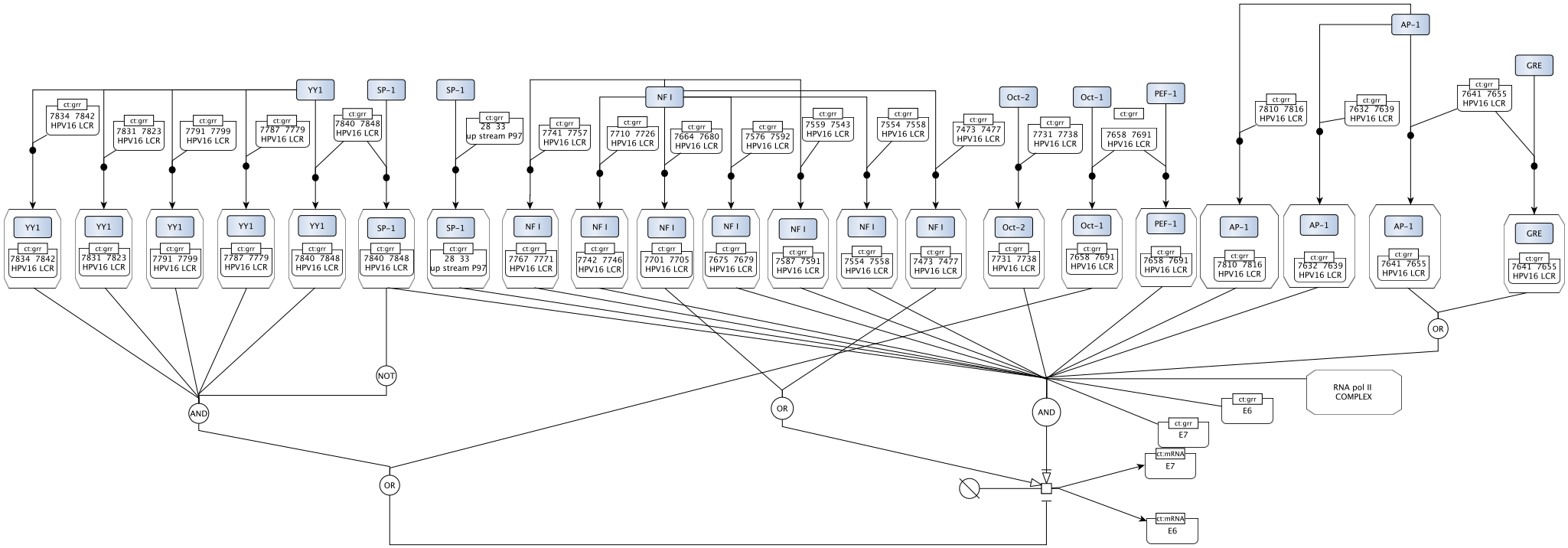

Figure 2: Constructed diagram of the transcription factors that bind to the long control region LCR of HPV16. Eight transcription factors and their effects are depicted in this map using The SBGN scheme. The binding sites of theses transcription factors occupy a region from 7454 to 7854 of HPV16 genome.

Transcription factors that are involved in the regulation of IFNγ gene expression

AP-1

Activator protein1 (AP-1) is a heterodimer nuclear protein consisting of the proto-oncogene products c-Jun and c-Fos. It is a very essential positive transcription activator of the cytokine genes which contain AP-1 activation elements in their promoters. AP-1 mediates positive transactivation through binding to DNA either independently or in association with NFAT. Activated AP-1 via interleukin18 (IL-18) induction can bind with the IFNγ promoter at a position from -196 to -183 independently. The induction ability of IL-18 is attributed to the ability of IL-18 to activate c-Jun N-terminal Kinase (JNK), which activates c-Jun (Kiani, 2001;Nakahira et al., 2002). Upon T cell activation, AP-1 binds at -163 to -155, but this binding requires the presence of NFATp. The third position is a primary NFAT weak binding site centred at -106 to -95, but NFATp only binds to the weak site in the presence of AP-1(Sweetser et al., 1998).

NFAT

The nuclear factor of activated T cells (NFAT) family is involved in the regulation of various inducible genes, especially those encoding cytokines and participating in the immune response (Rao, Luo, & Hogan, 1997;Kiani, 2001). T cells, particularly, express NFATp when in both the unstimulated and stimulated state; whereas NFATc is expressed primarily in activated T cells (Sweetser et al., 1998).

In the cytoplasm of resting T cells, NFATs are present as phosphoproteins and show low affinity for DNA. T cell receptor stimulation and calcium mobilization lead to activation of the calcium/calmodulin-dependent phosphatase calcineruin. This enzyme controls the translocation of NFAT proteins from the cytoplasm to the nucleus of activated cells through dephosphorylate NFAT proteins resulting in a substantial increase in NFAT affinity for DNA. Conversely, when calcineurin activity decreases due to inhibition via the removal of the calcium-mobilizing stimulus, NFAT proteins revert to their original phosphorylated state; thus, they return to the cytoplasm showing a low affinity for DNA (Rao, Luo, & Hogan, 1997;Kiani, 2001).

In the IFNγ promoter, NFATp binds strongly and independently to two regions spanning a position from -280 to -265 and from -163 to -155. The third NFATp binding sequence is at -106 to -91, but NFATp binds to this site weakly and only in the presence of AP-1 (Sweetser et al., 1998).

STAT4

Signal Transducer and Activator of Transcription (STAT) proteins are cytoplasmic proteins that induce distinct target genes. Activation of STAT4 in human T cells and natural killer (NK) cells relies on IFNα and IL-12 induction of a single tyrosine phosphorylation located around residue 700, which regulates the dimerization of STATs as a primary factor for the beginning of the classical JAK–STAT signalling pathway (Darnell Jr., 1997). Each of the IFNα and IL-12 cytokines also has the ability to induce serine phosphorylation of STAT4, mostly in the cytoplasm of the receptor. However serine phosphorylation does not show an essential role in the DNA binding of STAT4, but it could be necessary for optimal activation of STAT4-driven transcription by IL-12. That notion is based on experimental result where a mutation of S721 to alanine decreased the transcription factor activity of STAT4 when assayed in a transfected, IL-12-stimulation cell. Dimerized STAT4 proteins migrate to the nucleus, where they bind to the IFNγ promoter at position from -240 to -230bp. (Cho et al., 1996; Decker & Kovarik, 2000).

NF-κB proteins

The nuclear factor -κB (NF-κB) family proteins play a central role in controlling both innate and adaptive immunity responses and the regulation of cell survival and proliferation (Q. Li & Verma, 2002).(Santoro, Rossi, & Amici, 2003)

The NF-κB proteins are RELA (P65), NF-κB1 (P50), NF-κB2 (P52), c-Rel and RELB. P65 and P50 are expressed by various cell types, whereas RELB expression occurs in a specific region of the lymph nodes, thymus and Peyer's patches. Also, c-REL production is restricted to haematopoietic cells and lymphocytes (Q. Li & Verma, 2002).

In the cytoplasm of unstimulated cells, NF-κB proteins present as an inactive cytoplasmic complex due to binding to IκBα and IκBβ, which prevents NF-κB proteins from entering the nuclei(In, 2010) 2007). Upon viral infection, IκB kinase (IKK) production results in the activation of NF-κB via sequential phosphorylation and ubiquitination of IκB and subsequently, degradation of IκB by the proteasome (Mercurio, 1997). Free NF-κB, thus, can translocate to the nucleus and bind to its target DNA-binding site.

In the IFN-γ promoter, a binding site from -459 to -470 is recognized by the c-Rel protein. The second region from -772 to -763 is occupied by the NF-κB1(P50) homodimers, whereas the third position from -786 to 776 is bound by a heterodimer complex of NF-κB1(P50) and NF-κB2(P65), which is the main activated form of NF-κB (Sica et al., 1997; Li & Verma, 2002). The fourth position that is located between -278 and -268 has a homology sequence for NF-κB2(P65) and NFATp (Sweetser et al., 1998). There was a suggestion that the NF-κB-mediated induction of IFNγ promoter activity occurs primarily via two binding sites (-772 to -763) and (-786 to -776), whereas within the native IFNγ promoter the binding site from -278 to -268 is activated by calcineurin-inducible factors (Sica et al., 1997).

T-bet

T-bet is an essential transcription factor in the regulation of IFN-γ expression. Furthermore, it has been shown to recruit multiple mechanisms of action in controlling tissue-specific IFN-γ expression profiles (Young & Bream, 2007).

The IFN-γ promoter is methylated by a significant degree and serves as one means of repressing IFNγ expression in mature Th2 cells and in silencing the IFN-γ gene in Th1 cells. T-bet can bind to the IFN-γ promoter directly from -65 to -62 and can activate high-level IFN-γ gene expression despite the repressive epigenetic effect of CpG methylation at the IFNγ promoter in Th1 cells (Yu et al., 2006;Tong et all., 2005)

The ability of T-bet to induce IFN-γ gene expression in Th1 but not Th2 is attributed to the activated transcription factors such as NFAT, NF-kB and STAT that manage the activation or repression of subset-specific factors such as T-bet in developing Th1 cells, and GATA-3 in their Th2 counterparts . Moreover, T-bet itself can be induced by IFN-γ (Young et all., 2007;Tong et all., 2005).

T-bet phosphorylation is mediated by ITK at residue tyrosine 525 upon TCR stimulation. Furthermore, there was evidence that T-bet phosphorylation at residue serine 508 was not required for the ability of T-bet to bind DNA (Hwang et al., 2005).

c-Jun, ATF2 and CREB

C-Jun protein is very similar to viral protein that interacts with its target gene to regulate gene expression (www.ncbi.nlm.nih.gov). The transcription ability of the c-jun proto-oncogene appears by phosphorylation at residues serine 63 and serine 73 via c-jun amino-terminal kinase 2 (JNK2) (Kallunki et al., 1994).

ATF2 protein binds to the cAMP-responsive element (CRE) and it forms a homodimer or heterodimer with c-jun and induces CRE-dependent transcription. In addition, ATF2 protein specifically acetylates histones H2B and H4 in vitro. Thus, it may represent a class of sequence-specific factors which induce transcription through a direct effect on chromatin components (www.ncbi.nlm.nih.gov).
The transcription activity of ATF2 is induced via phosphorylation at residues threonine 69 and threonine 71 by stress-activated protein kinase/c-jun NH2-terminal kinase (SPAK/JNK), which is activated by many stimuli such as cellular stresses and extracellular signals(Livingstone et all., 1995;Nishina et all., 2004)

Cyclic AMP response element (CRE)-binding protein (CREB) is a transcription factor that is a member of the leucine zipper family of DNA binding proteins (www.ncbi.nlm.nih.gov). It is essential for cytokine production and T cell function. The transcriptional activity of CREB is augmented through phosphorylation at its residue serine 133 by different types of protein kinase (Shaywitz et all., 1999).

Upon T cell activation by *M. tuberculosis*, the three phosphorylated proteins (CREB, ATF-2 and c-Jun) form a complex and bind the IFNγ promoter at -73 to -48 (Samten et al., 2008).

There was a consensus on the positive effect of the heterodimer ATF-2 and c-Jun, but there was a contradiction in the role of CREB in IFNγ production. It has been demonstrated that CREB has a negative role in Jurkat T cells and transgenic mice (Penix et al., 1996; Aune et all., 1997). In contrast, CREB showed a positive role in primary T cells in response to *M. Tuberculosis (Samten et al., 2008 ; 2005)*. It was suggested that CREB has a positive traditional regulation role in latent but not active *M. tuberculosis* infection due to the diminished expression of CREB proteins. These conflicting results may attribute many different factors which can prevent the binding of CREB to its binding site in vivo resulting in reduction in IFNγ production, but these factors may not present under defined in vitro binding situation used for EMSA experiments (Yang Liu et al., 2010).

YYI

Ying-Yang1, YY1, a ubiquitous DNA-bending protein, is a zinc finger transcription factor that belongs to the human GLI-Krüppel family of nuclear proteins. YY1 protein the ability to up-regulate and down-regulate different gene expressions even in the same cell, depending upon the promoter (Ye et all., 1996). The four C-terminal GLI-Küppel type zinc fingers of YY1 have been identified as a transcriptional repression domain, particularly zinc finger 2, which plays a key role in both DNA bending and transcription repression (Galvin & Shi, 1997).

In the IFNγ promoter, three binding sites were revealed between positions -271 and -186. Of the three YY1 sites, one is in the silencer region from -232 to -219, which requires the formation of a complex of YY1 and AP-2like to perform the silencer activity. The second site is from -263 to -259, but it showed a very weak binding activity. The third site is between -205 and -200 where is close to the AP-1 binding site (-196 to -183). These two closed sites (from -211 to -186) cause competition between YY1 and AP-1 for binding in that region, despite their being sites do not overlap. The YY1 binding site (-232 to -219) might be dependent on the presence of the YY1 binding at -205 to -200. (Ye et all., 1996). Thus, since the YY1 concentration does not change upon cell stimulation in contrast to AP-1, we can understand the competition role between AP-1 and YY1 in IFNγ gene expression.

Transcription factors involved in the regulation of the LCR of HPV16 enhancement

AP-1 and glucocorticoid receptors

The long control region of HPV16 localizes two sequence motifs related to the heptamer, 5'-TGACTCA-3', that are bound by the transcription factor AP-1 (a heterodimer complex of c-jun and c-fos oncoproteins) from 7632 to 7639 and from 7641 to 7655. AP-1 binding sites may be

used by HPV16 in order to couple their gene regulation to downstream events of intracellular signalling. Is is activated by protein kinase C (PKC) that is induced by diacylglycerol, a second messenger stimulated by growth factors and certain polypeptide hormones (Chan et all., 1990).

The AP-1 binding site (7641 to 7655) includes a consensus sequence of the glucocorticoid responsive element GRE. Both of them have a positive effect on HPV16 early promoter activity but It is unknown whether the AP-1 protein and GRE bind to the AP-1 motif cooperatively or competitively (B.Gloss, H.U.Bernard, 1987). However, glucocorticoid responsiveness of HPV16 LCR partially requires the presence of NFI proteins binding to its binding sites (7675 to 7679) and (7701 to 7705), indicating that glucocorticoid receptors cooperate with NFI in transcriptional induction (Chan et all., 1990).

It was confirmed that GREs mediate the response to progesterone, which may have an important role in the HPV life cycle. Progesterone concentration increases during part of the ovulation and pregnancy cycle. These physiological changes could explain why women have more susceptibility to malignant HPV lesions than men, since HPV may benefit from the increased level of progesterone concentration (Chan et all., 1989).

NFI

Nuclear factor I (NFI) is individually capable of upregulation of viral gene expression and of viral DNA replication as well (Baldwin et all., 2007).

The LCR of HPV16 includes seven half palindromic sequence sites of 5'-TTGGC-3' that are bound by NFI . The binding sites are: NFI#1(7473 to 7477), NFI#2(7554 to 7558), NFI#3(7587 to 7591), NFI#4(7675 to 7679), NFI#5(7701 to 7705), NFI#6(7742 to 77446) and NFI#7(7767 to 7771). These binding sites have the ability to induce the enhancer of HPV16 in immortalized human keratinocytes in the absence of other LCR transcription factors. However, the contribution of each NFI binding site is not equal. Mutation of NFI sites #1 and #5 had very little effect on basal activity of the HPV16 LCR (Baldwin et all., 2007;Apt et all., 1993)

Oct-1 and PEF1

The Pou-domain transcription factor (Oct-1) is a ubiquitous nuclear transcription factor that is widely expressed in eukaryotic cells (Polanovsky, 2001). Oct-1 binds LCR HPV16 at a position from 7685 to 7691and negatively regulates the enhancer activity (Sibbet et all., 1995).

The same binding site (7685 to 7691) has a GC-rich sequence that is also bound by penta-EF-hand domain containing 1 (PEF-1). PEF-1 properties are not well known. It has a positive effect on the LCR of HPV16 although has a low affinity for its binding, suggesting that the nucleus has a low concentration of PEF-1 (Sibbet et all., 1995;Fergusson et all., 1998).

Generally, Oct-1 and PEF-1 bind to the same site and act on the LCR of HPV16 in opposite manners but there was no clear evidence of the two factors cooperating or competing for binding.

Oct-2

LCR of HPV16 includes an octamer binding site from 7731 to 7738. This binding site contains a sequence which includes a seven-out-of-eight base match to the consensus binding site for Oct-1. In contrast, the LCR of the non-tumorigenic human papillomaviruses lack the octamer binding site. Cervical cells express, in addition to Oct-1, the tissue-specific octamer binding protein Oct-2, that is also found in B cells, neuronal cells and the testis, but most other cells lack this protein (D. Latchman, 1996;Morris et all., 1993).

Oct-2 binds the LCR of HPV16 at 7731 to 7738 and enhances promoter activity in contrast to OCT-1, indicating that Oct-2 may have a role in the cervical specificity of the LCR by binding transactivation octamer binding protein Oct-2, which is produced in cervical cells but is absent in other cell types. The difference in function of Oct-1 and Oct-2 on HPV16 is attributed to the differences in both the N and C terminal domains (Morris et all., 1993).

SP-1

SP-1 has been implicated in the regulation of the expression of a variety of genes involved in several biological processes including immune response and apoptosis (uniport.org). Thus, cells lacking SP-1 have little chance of survival (Philipsen et all., 1999). It has been demonstrated that SP-1 interacts physically with the E2 protein of the human papillomaviruses (Peng et all., 2003), which regulates the activity of the P97 promoter in HPV16.

In the LCR of HPV16, two binding sites for SP-1 have been identified. The first binding site is at 28 to 33 upstream of the P97 promoter, which suggests that it is essential for the early transcription start of the E6/E7 promoter of HPV16 (Gloss & Bernard, 1990). The second SP-1 binding site is located from 7842 to 7847 which overlaps one of the YY1 binding sites at a position from 7840 to 7848. It was concluded that SP-1 and YY1 compete for DNA binding at a position 7840–7848, each of which has the opposite effect on LCR HPV16 activation. YY1 shows more

affinity for DNA binding than SP-1 when the concentrations of both proteins are the same such as in normal keratinocytes which have a low concentration of SP-1. In contrast, SP-1 concentration increases in transformed keratinocytes (tumour cells) and in differentiated ones (Dong et all., 1999). Thus, SP-1 binds more efficiently than YY1 and increases the activity of the P97 promoter. As a result, the concentration of YY1 and SP-1 may contribute to the tissue specification of HPV16.

YY1

As mentioned previously, YY1 is a multifunctional transcription factor that plays a role in regulating the gene expression of several cellular and viral genes. Within the HPV16 LCR (from 7401 to 103), it was found that there were 5 binding sites out of 24 binding sites were recognized by YY1 and that have the ability to repress LCR enhancer activity (O'Connor et all, 1996). All 5 sites are essential for transcription repressing of LCR of HPV16. These positions are located in (7834 to 7842), (7831 to 7823), (7791 to 7799), (7787 to 7779) and (7840 to 7848). SP-1 competes with YY1 to bind at latter sites (Dong et all., 1999).

It was concluded that YY1 represses HPV16 promoter activity through competition with SP-1 for DNA binding at 7840 to 7848 and through quenching AP-1 activity because 4 of the 5 YY1 sites are located around its binding site (7810 to 7816). Nevertheless, since the 5 YY1 sites are required for repressing HPV16 activity, it was found that a number of metastases or primary tumours contained HPV16 episomes with deletions or point mutations at one or more of the YY1 binding sites; thus, HPV16 can escape form cellular repression via YY1 (May et al., 1994).

Partner 1 Gene Symbol	Partner 1 Entrez Gene Id	Partner 1 Molecule Type	Partner 1 Molecule Type Ontology Reference	Partner 2 Gene Symbol	Partner 2 Entrez Gene Id	Partner 2 Molecule Type	Partner 2 Molecule Type Ontology Reference	Interaction Type	Interaction Type Ontology Reference	Location	Location Ontology Reference	Pubmed ID	Species	Cell Type	Technique	Technique Ontology Reference	Pathway
STAT4	6775	Protein	MI:0326	IFN-g -230 to -240	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	9558063/11449378	Human	T-cell/NK cell	EMSA/ensa supershift/western blotting	MI:0413/MI:0412/MI:0113	IFNg expression
T-bet	30009	Protein	MI:0326	IFN-g -65 5'-TGTG-3' -62	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	15684083/16713975/15862016	Human	T-cell/NK cell	EMSA/western blotting	MI:0413/MI:0113	IFNg expression
C-Jun	3725	Protein	MI:0326	IFN-g -197 5'-TGTCTCA-3' -190	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	8756632/notte	Human	T-cell	EMSA/ensa supershift	MI:0413/MI:0412	IFNg expression
YY1	7528	Protein	MI:0326	IFN-g -205 5'-AATGGG-3' -200	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	8756632	Human	T-cell	EMSA	MI:0413	IFNg expression
YY1	7528	Protein	MI:0326	IFN-g -232 5'-CCCACCAGAATGGC-3' -219	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	8756632	Human	T-cell	EMSA	MI:0413	IFNg expression
NF-kB/P50	4790	Protein	MI:0326	IFN-g -786 5'-GGGGAGTTCC-3' -776	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	12773372/9374532	Human	T-cell	EMSA supershift	MI:0412	IFNg expression
NF-kB/P65	5970	Protein	MI:0326	IFN-g -786 5'-GGGGAGTTCC-3' -776	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	12773372/9374532	Human	T-cell	EMSA supershift	MI:0412	IFNg expression
NF-kB/P50	4790	Protein	MI:0326	IFN-g -772 5'-AAAATATTCC-3' -763	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	12773372/9374532	Human	T-cell	EMSA supershift	MI:0412	IFNg expression
NF-kB/P65	5970	Protein	MI:0326	IFN-g -260 -265	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	12773372/9374532	Human	T-cell	EMSA supershift	MI:0412	IFNg expression
NF-kB/c-Rel	5966	Protein	MI:0326	IFN-g 459 470	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	12773372/9374532/1542667	Human	T-cell	EMSA supershift	MI:0412	IFNg expression
NFAT1	4773	Protein	MI:0326	IFN-g -168 -153	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	9857002/9143705	Human	T-cell	EMSA/Footprinting	MI:0413/MI:0417	IFNg expression
NFAT1	4773	Protein	MI:0326	IFN-g -280 -265	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	9857002/9143705	Human	T-cell	EMSA/Footprinting	MI:0413/MI:0417	IFNg expression
NFAT1	4773	Protein	MI:0326	IFN-g -106 -95	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	9857002/9143705	Human	T-cell	EMSA/Footprinting	MI:0413/MI:0417	IFNg expression
c-Jun	3725	Protein	MI:0326	IFN-g -168 -153	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	9857002/9143705	Human	T-cell	EMSA/Footprinting	MI:0413/MI:0417	IFNg expression
c-Jun	3725	Protein	MI:0326	IFN-g -106 -95	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	9857002/9143705	Human	T-cell	EMSA/Footprinting	MI:0413/MI:0417	IFNg expression
ATF2	1386	Protein	MI:0326	IFN-g -73 -48	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	18641343	Human	T-cell	EMSA/ensa supershift/coimmunoprecipitation/western blotting	MI:0413/MI:0412/MI:0019/MI:0113	IFNg expression
C-Jun	3725	Protein	MI:0326	IFN-g -73 -48	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	18641343	Human	T-cell	EMSA/ensa supershift/coimmunoprecipitation/western blotting	MI:0413/MI:0412/MI:0019/MI:0113	IFNg expression
CREB	1385	Protein	MI:0326	IFN-g -73 -48	3458 Promoter region	promoter region	SO:0000832	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	18641343	Human	T-cell	EMSA/ensa supershift/coimmunoprecipitation/western blotting	MI:0413/MI:0412/MI:0019/MI:0113	IFNg expression

Table 1: Details and references for the interactions shown in Figure 1.

Partner 1 Gene Symbol	Partner 1 Entrez Gene Id	Partner 1 Molecule Type	Partner 1 Molecule Type Ontology Reference	Partner 2 Gene Symbol	Interaction Type	Interaction Type Ontology Reference	Location	Location Ontology Reference	Pubmed ID	Species	Cell Type	Technique	Technique Ontology Reference	Pathway
AP-1(c-jun)	3725	Protein	MI:0326	HPV16/LCR 7632 7639	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	1318197/2545902/2536825	Human	Human keratinocute & HaCat /Hela cell	footprinting/point mutation	MI:0417/	HPV16/LCR activation
AP-1(c-jun)	3725	Protein	MI:0326	HPV16/LCR 7641 7655	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	1318197/2545902/2536825	Human	Human keratinocute & HaCat /Hela cell	footprinting/point mutation	MI:0417/	HPV16/LCR activation
AP-1(c-jun)	3725	Protein	MI:0326	HPV16/LCR 7810 7816			nucleus	GO:0005634	2536825/8794287	Human	HeLa cells/Human keratinocyte	footprinting/EMSA	MI:0417/MI:0413	HPV16/LCR activation
GRE		Protein	MI:0326	HPV16/LCR 7641 7655	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	1318197/2545902/2536825	Human	Human keratinocute & HaCat /Hela cell	footprinting/point mutation	MI:0417/	HPV16/LCR activation
OCT-1	5451	Protein	MI:0326	HPV16/LCR 7658 7691	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	7769658	Human	HeLa, SiHa, and MRC-5	EMSA	MI:0413	HPV16/LCR activation
PEF-1	553115	Protein	MI:0326	HPV16/LCR 7658 7691	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	7769658	Human	HeLa, SiHa, and MRC-5	EMSA	MI:0413	HPV16/LCR activation
OCT-2	5452	Protein	MI:0326	HPV16/LCR 7731 7738	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	8233784/8383834	Human	Namalwa B cells/BHK fibroblasts/CaSki cervical cells/Hela	EMSA	MI:0413	HPV16/LCR activation
NFI	4782	Protein	MI:0326	HPV16/LCR 7473 7477	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	2542901/19440457/2536825/15047811	Human	HeLa	footprinting/electroporation	MI:0417/MI:0308	HPV16/LCR activation
NFI	4782	Protein	MI:0326	HPV16/LCR 7554 7558	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	2542901/19440457/2536825/15047811	Human	HeLa	footprinting/electroporation	MI:0417/MI:0308	HPV16/LCR activation
NFI	4782	Protein	MI:0326	HPV16/LCR 7587 7591	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	2542901/19440457/2536825/15047811	Human	HeLa	footprinting/electroporation	MI:0417/MI:0308	HPV16/LCR activation
NFI	4782	Protein	MI:0326	HPV16/LCR 7675 7679	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	2542901/19440457/2536825/15047811	Human	HeLa	footprinting/electroporation	MI:0417/MI:0308	HPV16/LCR activation
NFI	4782	Protein	MI:0326	HPV16/LCR 7701 7705	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	2542901/19440457/2536825/15047811	Human	HeLa	footprinting/electroporation	MI:0417/MI:0308	HPV16/LCR activation
NFI	4782	Protein	MI:0326	HPV16/LCR 7742 7746	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	2542901/19440457/2536825/15047811	Human	HeLa	footprinting/electroporation	MI:0417/MI:0308	HPV16/LCR activation
NFI	4782	Protein	MI:0326	HPV16/LCR 7767 7771	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	2542901/19440457/2536825/15047811	Human	HeLa	footprinting/electroporation	MI:0417/MI:0308	HPV16/LCR activation
SP-1	6667	Protein	MI:0326	HPV16/LCR 7840 7848	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	10466808	Human	HeLa	EMSA	MI:0413	HPV16/LCR activation
SP-1	6667	Protein	MI:0326	HPV16 28 33 upstream P97	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	2170687	Human	HeLa, SiHa, and CaSKi cells	Footprinting	MI:0417	HPV16/LCR activation
YY1	7528	Protein	MI:0326	HPV16/LCR 7840 7848	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	8137827/8794287	Human	(SiHa/HT3/CaSki)(Human keratinocyte/Hela cells)	Southern blot/EMSA/Footprinting	MI:0103/MI:0413/MI:0417	HPV16/LCR activation
YY1	7528	Protein	MI:0326	HPV16/LCR 7787 7779	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	8137827/8794287	Human	(SiHa/HT3/CaSki)(Human keratinocyte/Hela cells)	Southern blot/EMSA/Footprinting	MI:0103/MI:0413/MI:0417	HPV16/LCR activation
YY1	7528	Protein	MI:0326	HPV16/LCR 7791 7799	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	8137827/8794287	Human	(SiHa/HT3/CaSki)(Human keratinocyte/Hela cells)	Southern blot/EMSA/Footprinting	MI:0103/MI:0413/MI:0417	HPV16/LCR activation
YY1	7528	Protein	MI:0326	HPV16/LCR 7831 7823	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	8137827/8794287	Human	(SiHa/HT3/CaSki)(Human keratinocyte/Hela cells)	Southern blot/EMSA/Footprinting	MI:0103/MI:0413/MI:0417	HPV16/LCR activation
YY1	7528	Protein	MI:0326	HPV16/LCR 7843 7842	sequence specific DNA binding	GO:0043565	nucleus	GO:0005634	8137827/8794287	Human	(SiHa/HT3/CaSki)(Human keratinocyte/Hela cells)	Southern blot/EMSA/Footprinting	MI:0103/MI:0413/MI:0417	HPV16/LCR activation

Table 2: Details and references for the interactions shown in Figure 2.

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