

Latent infection and abnormal cell proliferation

By

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WP 1005

November 7, 2002

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I. The misconception

The current view holds that viral proteins are the sole mediators of viral effects on the host cell. In simple terms, “no viral protein, no effect.” No protein, or almost no protein, is a characteristic of a latent viral infection. Therefore, the current view holds that a latent viral infection is mostly harmless. This report presents the discovery of a “protein-independent” effect of viral DNA on cellular gene expression. The report shows that, under certain conditions, competition between viral and cellular DNA can disrupt the transcription of cellular genes, which, in turn, can lead to reduced cell differentiation and excessive cell proliferation. Moreover, the report also shows that the protein-independent effect can explain the abnormal transcription of many genes in cancer, specifically, the reduced transcription of BRCA1 and Fas.

II. The discovery

A. Background

1. GABP binds many cellular genes

The transcription factor GA Binding Protein, or GABP (also called Nuclear Respiratory Factor 2 (NRF-2)¹, E4 Transcription factor 1 (E4TF1)¹ and Enhancer Factor 1A (EF-1A)²) binds the promoter and enhancers of many cellular genes, including BRCA1 (Atlas 2000²), Fas (Li 1999³), interleukin 16 (IL-16) (Bannert 1999⁴), interleukin 2 (IL-2) (Avots 1997⁵), interleukin 2 receptor β -chain (IL-2R β) (Lin 1993⁶), IL-2 receptor γ -chain (IL-2 γ c) (Markiewicz 1996⁷), human secretory interleukin-1 receptor antagonist (secretory IL-1ra) (Smith 1998⁸), retinoblastoma (Rb) (Sowa 1997⁹), human thrombopoietin (TPO) (Kamura 1997¹⁰), aldose reductase (Wang 1993¹¹), neutrophil elastase (NE) (Nuchprayoon 1999¹², Nuchprayoon 1997¹³), folate binding protein (FBP) (Sadasivan 1994¹⁴), cytochrome c oxidase subunit Vb (COXVb) (Basu 1993¹⁵, Sucharov 1995¹⁶), cytochrome c oxidase subunit IV (Carter 1994¹⁷, Carter 1992¹⁸), mitochondrial transcription factor A (mtTFA) (Virbasius 1994¹⁹), β subunit of the FoF1 ATP synthase (ATPsyn β) (Villena 1998²⁰), prolactin (PRL) (Ouyang 1996²¹) and the oxytocin receptor (OTR) (Hoare 1999²²) among others. For some of these genes, for instance, CD18, COXVb, COXIV, GABP binds to the promoter while for others, for example IL-2 and ATPsyn β , GABP binds an enhancer.

2. Viral DNA also binds GABP

The N-box, the DNA box which binds GABP, is the core binding sequence of many viral enhancers, including the polyomavirus enhancer area 3 (PEA3) (Asano 1990²³), adenovirus E1A enhancer (Higashino 1993²⁴), Rous Sarcoma Virus (RSV) enhancer (Lamins 1984²⁵), Herpes Simplex Virus 1 (HSV-1) (in the promoter of the immediate early gene ICP4) (LaMarco 1989²⁶, Douville 1995²⁷), Cytomegalovirus (CMV) (IE-1

¹ Nuclear Respiratory Factor 2 should not be confused with NF-E2 Related Factor 2 which is also abbreviated NRF2 or NRF-2.

² Enhancer Factor 1A should not be confused with Elongation Factor 1A which is also abbreviated EF-1A.

enhancer/promoter region) (Boshart 1985²⁸), Moloney Murine Leukemia Virus (Mo-MuLV) enhancer (Gunther 1994²⁹), Human Immunodeficiency Virus (HIV) (the two NF- κ B binding motifs in the HIV LTR) (Flory 1996³⁰), Epstein-Barr virus (EBV) (20 copies of the N-box in the +7421/+8042 oriP/enhancer) (Rawlins 1985³¹) and Human T-cell lymphotropic virus (HTLV) (8 N-boxes in the enhancer (Mauclere 1995³²) and one N-box in the LTR (Kornfeld 1987³³)). Note that some viral enhancers, for example SV40, lack a precise N-box but still bind the GABP transcription factor (Bannert 1999, *ibid*).

Ample evidence exists supporting binding of GABP to the N-boxes in these viral enhancers. For instance, Flory 1996 (*ibid*) shows binding of GABP to the HIV LTR, Douville 1995 (*ibid*) shows binding of GABP to the promoter of ICP4 of HSV-1, Bruder 1991³⁴ and Bruder 1989³⁵ show binding of GABP to the adenovirus E1A enhancer element I, Ostapchuk 1986³⁶ shows binding of GABP (called EF-1A in the paper) to the polyomavirus enhancer, and Gunther 1994 (*ibid*) shows binding of GABP to Mo-MuLV. Other studies demonstrate competition between the above viral enhancers and enhancers of other viruses. For instance, Scholer and Gruss (1984³⁷) show competition between the Moloney Sarcoma Virus (MSV) enhancer and SV40 enhancer, and competition between the RSV enhancer and the BK virus enhancer.

3. The GABP•p300/cbp transcription complex is limiting

The coactivator p300 is a 2,414-amino acid protein initially identified as a binding target of the E1A oncoprotein. cbp is a 2,441-amino acid protein initially identified as a transcriptional activator bound to phosphorylated cAMP response element (CREB) binding protein (hence, cbp). p300 and cbp share 91% sequence identity and are functionally equivalent. Both p300 and cbp are members of a family of proteins collectively referred to as p300/cbp.

Although p300/cbp are widely expressed, their cellular availability is limited. Several studies demonstrated inhibited activation of certain transcription factors resulting from competitive binding of p300/cbp to other cellular or viral proteins. For example, competitive binding of p300, or cbp, to the glucocorticoid receptor (GR), or the retinoic acid receptor (RAR), inhibited activation of a promoter dependent on the AP-1 transcription factor (Kamei 1996³⁸). Competitive binding of cbp to STAT1 α inhibited activation of a promoter dependent on both the AP-1 and *ets* transcription factors (Horvai 1997³⁹). Competitive binding of p300 to STAT2 inhibited activation of a promoter dependent on the NF- κ B RelA transcription factor (Hottiger 1998⁴⁰). Other studies also demonstrated limited availability of p300/cbp, see, for instance, Pise-Masison 2001⁴¹, Banas 2001⁴², Wang 2001⁴³, Ernst 2001⁴⁴, Yuan 2001⁴⁵, Ghosh 2001⁴⁶, Li 2000⁴⁷, Nagarajan 2000⁴⁸, Speir 2000⁴⁹, Chen 2000⁵⁰, and Werner 2000⁵¹.

GABP binds p300/cbp (Bannert 1999, *ibid*). Since p300/cbp is limiting, the transcription complex GABP•p300/cbp is also limiting.

B. Microcompetition

Consider a persistent latent infection with a virus that binds GABP•p300/cbp (see list of viruses above). Can such an infection disrupt the transcription of a cellular gene?

Take BRCA1 for example. BRCA1 binds GABP•p300/cbp. Binding of GABP•p300/cbp transactivates the gene and increases the concentration of the BRCA1 protein in the cell (see above). GABP•p300/cbp is limiting (see above). By binding GABP•p300/cbp, the virus decreases availability of the complex to the BRCA1 promoter, which decreases binding of GABP•p300/cbp to the promoter. The result is a decrease in BRCA1 transcription.

Competition between a viral N-box and the promoter/enhancer of a cellular GABP regulated gene results in disrupted transcription of the cellular gene. If GABP•p300/cbp stimulates transcription of the cellular gene, competition with the viral N-box inhibits transcription. If the complex suppresses transcription, the competition stimulates transcription.

Consider the effect of an increase in the copy number of the viral N-boxes in the nucleus. The increase further decreases availability of the GABP•p300/cbp complex to the cellular gene, which further decreases, or increases, transcription depending on the effect of GABP•p300/cbp on transcription.

Terminology:

1. A virus that binds GABP•p300/cbp is called a GABP virus.
2. Competition between two polynucleotides for the same transcription factor is called microcompetition.
3. A polynucleotide that binds GABP•p300/cbp is called an N-box. For instance, the polynucleotide AGGAAG binds GABP•p300/cbp.

Notations:

Let “↑,” “↓” denote increase and decrease, respectively.

Let “→” denote “leads to.”

Let “g” denote a cellular GABP•p300/cbp stimulated gene.

Let “[N-box_v]” denote the copy number of a viral N-box in a cell.

The effect of the copy number of a viral N-box in a cell on g transcription can be presented symbolically as follows (assume that g is a GABP•p300 stimulated gene):

$$\uparrow[\text{N-box}_v] \rightarrow \downarrow[\text{mRNA}_g]$$

Note that the same reasoning holds for every limiting transcription complex that binds both viral and cellular, or foreign and natural DNA.

III. Predictions and observations

To test the concept of microcompetition, the following sections present logical conclusions that describe the predicted effects of microcompetition with a viral polynucleotide on cellular gene transcription and cell function. The logical predictions are compared to observations reported in the literature. In all cases, the observations are consistent with the predicted effects, and therefore, validate the new concept.

All studies in the following section used the same experimental design. The study objective was to test the effect of a certain gene, viral or cellular, on cell function. To perform the test, the studies inserted the gene of interest into a standard plasmid and then transfected the “test gene” plasmid into certain cells. As a control, the studies used cells transfected with the “empty” vector, that is, the standard plasmid without the gene of interest, or non-transfected, “wild-type” cells.

It is interesting that all studies compared, as expected, the test gene transfected cells to the empty vector transfected cells and to the wild-type cells. However, no study compared the empty vector transfected cells to the wild-type cells. See figure 1.

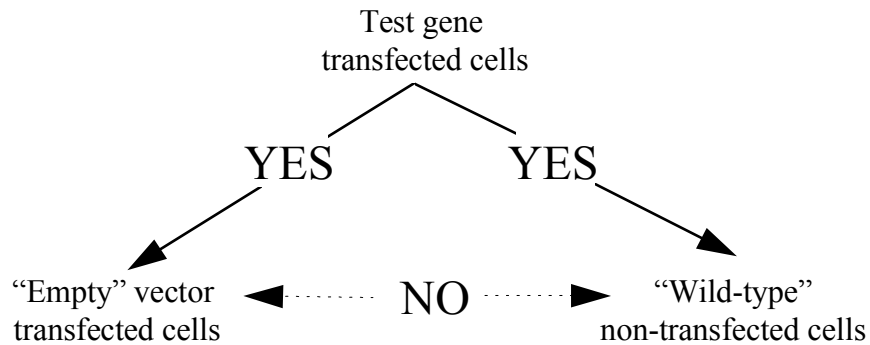


Figure 1: The experimental design of the cited studies

A. Cherington 1988

A study (Cherington 1988⁵²) inserted the wild-type early region of SV40, which expresses the SV40 large T antigen, into the pZIP-Neo plasmid (the test gene plasmid). The study transfected 3T3-F442A preadipocytes with either the test gene or the “empty” pZIP-neo plasmid. Some cells were not transfected (wild-type, WT) (note that, in the paper, the test gene plasmid, and not a non-transfected cell, is labeled “wild-type”). Accumulation of triglyceride, assayed by oil red staining, was used as a marker of differentiation. Seven days post confluence, the study recorded the staining of cells.

pZIP-neo expresses the neomycin-resistance gene under control of the Moloney murine leukemia virus long terminal repeat (LTR) (Cepko 1984⁵³). The LTR binds GABP (see

above). Rb is a GABP stimulated gene (see above). Assume that a decrease in Rb expression reduces cellular differentiation. According to microcompetition:

$$\uparrow[\text{pZIP-Neo}] \rightarrow \downarrow[\text{N-box}_{\text{Rb}} \bullet \text{GABP} \bullet \text{p300/cbp}] \rightarrow \downarrow[\text{mRNA}_{\text{Rb}}] \rightarrow \downarrow[\text{triglyceride}]$$

Transfection with the “empty” vector, pZIP-neo, should reduce accumulation of triglycerides relative to non-transfected cells. ([triglyceride] denotes the concentration of triglycerides.)

Consider figure 2 (based on Cherington 1988, Fig 4 A, B and C). Darker staining indicates increased differentiation.

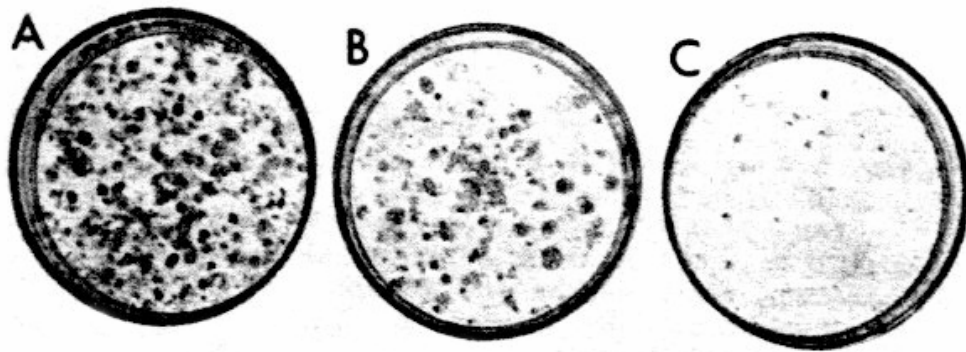


Figure 2: Adipocyte differentiation in (A) non-transfected F442A cells (WT control), (B) cells transfected with pZIP-neo (“empty” vector control), (C) cells transfected with the test gene plasmid. (Reproduced with permission granted by the American Society for Microbiology, and by the author, Dr. Van Cherington).

Transfection with the test gene plasmid, the vector expressing the SV40 large T antigen, reduced differentiation, compare triglyceride staining in C and A. Transfection with the “empty” vector, although less effective than the test gene vector, also reduced differentiation. Compare triglyceride staining in B relative to A and C. The results are consistent with the predicted effect of microcompetition.

B. Higgins 1996

A study (Higgins 1996⁵⁴) transfected murine 3T3-L1 preadipocytes with PVU0, a vector that carries an intact early region of the SV40 genome, which includes the SV40 large and small tumor antigens (the test gene plasmid). The cells were also transfected with HSV-neo, or pZIP-neo, as “empty” controls. Following transfection, the study cultured the cells under differentiation inducing conditions, and measured glycerophosphate dehydrogenase (GPD) activity as a marker of differentiation.

HSV-neo is a plasmid that expresses the neomycin-resistance gene under control of the murine Harvey sarcoma virus long terminal repeat (LTR) (Armelin 1984⁵⁵). pZIP-neo expresses the neomycin-resistance gene under control of the Moloney murine leukemia virus (MMLV) long terminal repeat (LTR) (Cepko 1984, *ibid*). Both the LTRs bind

GABP (see above). Assume that a decrease in mRNA_{Rb} reduces both cell arrest and differentiation. According to microcompetition:

$$\uparrow[\text{HSV-neo}] \text{ OR } \uparrow[\text{pZIP-neo}] \rightarrow \downarrow[\text{N-box}_{\text{Rb}} \bullet \text{GABP} \bullet \text{p300}] \rightarrow \downarrow[\text{mRNA}_{\text{Rb}}] \rightarrow \downarrow[\text{GPD}]$$

Transfection with the “empty” vectors, HSV-neo or pZIP-neo, should reduce cell differentiation relative to non-transfected cells (WT control) ([GPD] denotes GPD activity.)

The results are presented in table 1 (Higgins 1996, *ibid*, Table 1, first four lines).

Vector	Cell line	GPD activity (U/mg of protein)
None (WT control)	L1	2,063 1,599
HSV-neo (“empty” vector control A)	L1-HNeo	1,519 1,133
pZIP-neo (“empty” vector control B)	L1-ZNeo	1,155 1,123
PVU0 (test gene)	L1-PVU0	47 25
P value (EV-HSV vs. WT)		0.118
P value (EV-ZIP vs. WT)		0.103

Table 1

Transfection with PVU0, which expresses the large and small T antigens, resulted in a statistically significant decrease in GPD activity. Transfection of the “empty” vectors, HSV-neo and ZIP-neo, although less effective than PVU0, also reduced GPD activity. In a t-test, assuming unequal variances, the p-value for the difference between the HSV-neo vector and no vector is 0.118, and the p-value for the difference between ZIP-neo and no vector is 0.103. Given that the sample includes only two observations, a p-value around 10% for vectors carrying two different LTRs indicates a trend. The observations demonstrate the effect of the “empty” vectors HSV-neo and Zip-Neo on cell differentiation. The results are consistent with the predicted effect of microcompetition.

C. Awazu 1998

A study (Awazu 1998⁵⁶) transfected HuH-7 human hepatoma cells with pBARB, a plasmid that expresses the Rb gene under the control of the β -actin promoter (hence, the BA RB in the name), and expresses the neomycin-resistance (neo) gene under the control of the simian virus (SV40) promoter. The study also transfected cells with the pSV40-neo plasmid, which only includes the SV40 promoter and the neo gene. Since pSV40-neo does not include the β -actin promoter and Rb gene, the study considered the pSV40-

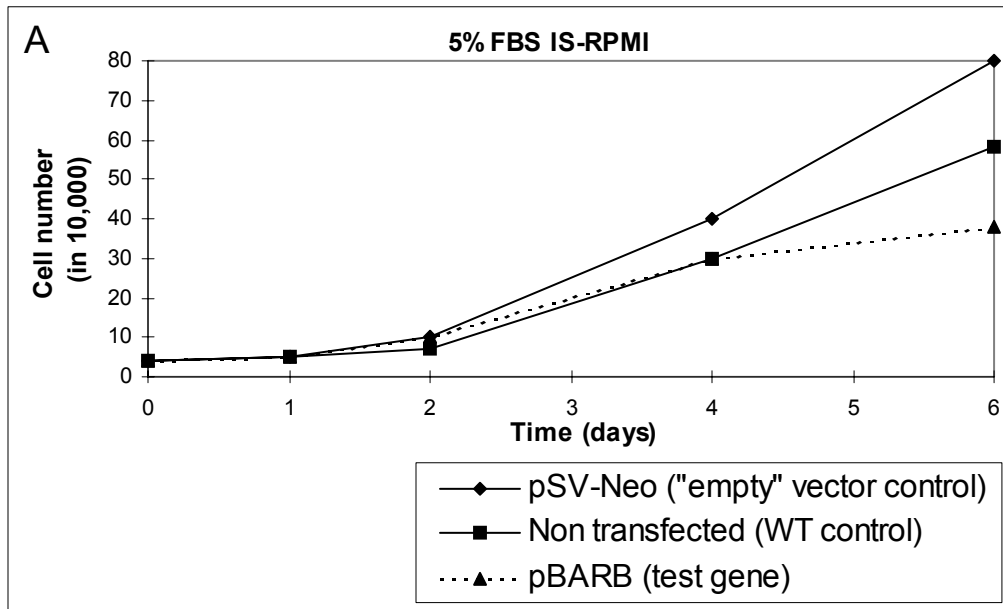
neo plasmid as “empty” and used it as a control. The cells were incubated in IS-RPMI, with or without 5% FBS, and the number of viable cells was counted at the indicated times.

The “empty” vector includes the SV40 promoter that binds GABP•p300/cbp. Therefore, according to microcompetition:



Transfection with the “empty” vector should increase the number of viable cells compared to non-transfected cells (WT), that is, it should induce cell proliferation.

Figures 3A and 3B summarize the results (based on Awazu 1998, *ibid*, Fig 2A). The SD is about the size of the triangular/rectangular symbols.



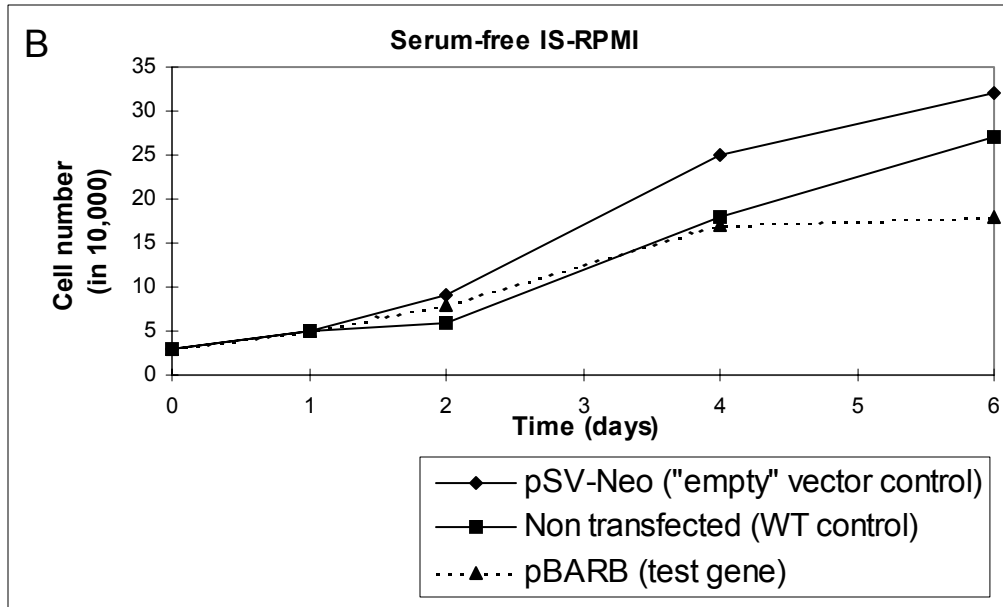


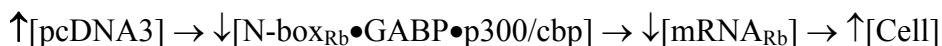
Figure 3: Growth of non-transfected cells, cells transfected with the pSV-Neo “empty” vector, and cells transfected with pBARB, the test gene plasmid. (A) Cells incubated in IS-RPMI with 5% FBS. (B) Cells incubated in serum free IS-RPMI.
(Reproduced with permission granted by the Academic Press)

The results demonstrate the effect of transfection with pSV40-neo, the “empty” vector, on cell proliferation. The results are consistent with predicted effect of microcompetition.

D. Choi 2001

Another study (Choi 2001⁵⁷) stably transfected the human multiple myeloma (MM)-derived cell line ARH with the pcDNA3 plasmid carrying an antisense to the macrophage inflammatory protein 1- α (MIP-1 α) (AS-ARH) (the test gene plasmid). As a control, the study transfected other ARH cells with the “empty” pcDNA3 vector (EV-ARH). To measure the effect of the antisense on cell growth, the study cultured 10^5 non-transfected (WT control), pcDNA3 (“empty” vector control), and MIP-1a antisense (test gene plasmid) transfected ARH cells in six-well plates with RPMI-1640 media containing 10% FBS. At days 3 and 5, the cells were sampled, stained and counted.

The pcDNA3 vector carries the cytomegalovirus (CMV) promoter that binds GABP•p300/cbp. According to microcompetition:



Transfection with the “empty” vector should increase the number of viable cells compared to non-transfected cells, that is, it should induce cell proliferation. Consider figure 4 (Choi 2001, *ibid*, Fig. 2a).

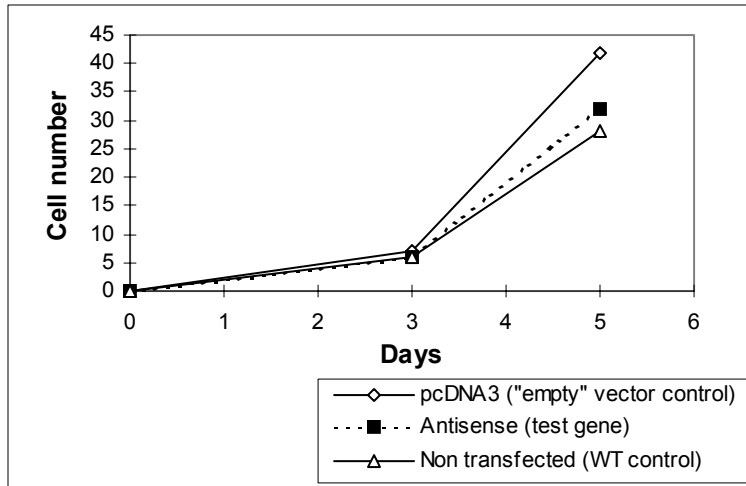


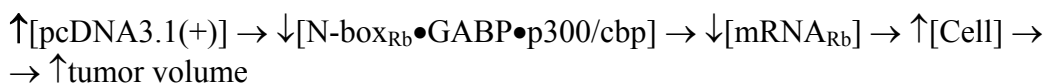
Figure 4: Growth of non-transfected cells, cells transfected with the pcDNA3 “empty” vector, and cells transfected with the antisense sequence, the test gene plasmid. (Reproduced with permission granted by the Copyright Clearing Center, Inc.)

As expected, after 5 days in culture, the number of cells transfected with the “empty” vector was larger than the number of non-transfected cells. The results are consistent with the predicted effect of microcompetition.

E. Hu 2001

Another study (Hu 2001⁵⁸) measured the efficacy and safety of an immunoconjugate (icon) molecule, composed of a mutated mouse factor VII (mfVII) targeting domain, and the Fc effector domain of an IgG1 Ig (mfVII/Fc icon), in the severe combined immunodeficient (SCID) mouse model of human prostatic cancer. First, the study injected the mice s.c. in both rear flanks with the human prostatic cancer line c4-2. The injection resulted in skin tumors. On days 0,3,6,9,12,15,33,36,39, and 42, the study injected into the skin tumor on one flank, the pcDNA3.1(+) vector carrying the icon (four mice), or the “empty” vector (four mice). The tumor on the other flank was left uninjected. The study measured tumor volume in the injected and non-injected flanks.

The pcDNA3.1(+) vector carries the cytomegalovirus (CMV) promoter that binds GABP•p300/cbp. According to microcompetition:



Injection of the “empty” vector transfected cells should increase the volume of the injected tumors compared to uninjected tumors. Consider figure 5 (Hu 2001, *ibid*, Fig 3).

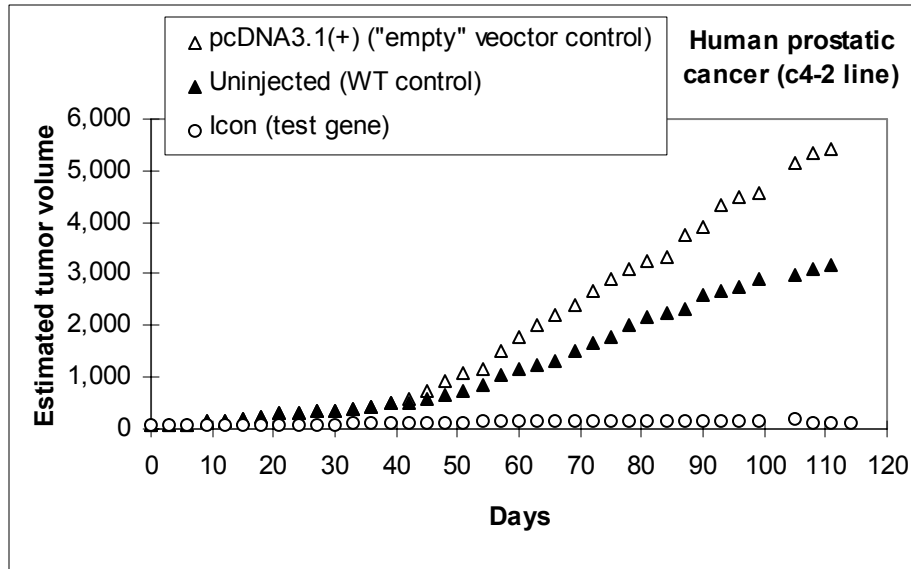


Figure 5: Growth of: ○- tumors injected with the icon vector (test gene), Δ- tumors injected with pcDNA3.1(+) (“empty” vector control), ▲- uninjected tumors on the other flank in pcDNA3.1(+) injected mice (WT control).

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As expected, tumors injected with the “empty” vector transfected cells showed higher volumes compared to uninjected tumors.

The experiment was repeated with the human melanoma line TF2 instead of the human prostatic cancer line C4-2. The results are presented in figure 6 (Hu 2001, *ibid*, Fig. 5)

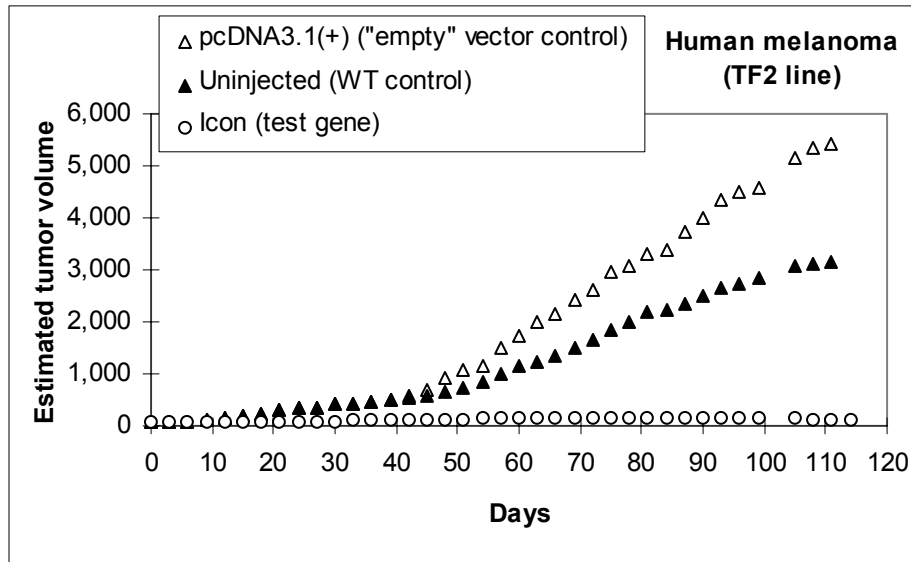


Figure 6: Growth of: ○- tumors injected with the icon vector (test gene), Δ- tumors injected with pcDNA3.1(+) (“empty” vector control), ▲- uninjected tumors on the other flank in pcDNA3.1(+) injected mice (WT control).

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In both experiments, injection of the “empty” vector stimulated tumor growth. Compare (Δ) - tumors injected with the “empty” vector and (\blacktriangle) - uninjected tumors in the “empty” vector injected mice (WT control). In a t-test, assuming unequal variances, the p-values for the difference between tumors injected with the “empty” vector (Δ) and uninjected tumors (\blacktriangle), in both experiments, is 0.0265, which is considered statistically significant. The results are consistent with the predicted effect of microcompetition.

F. Summary

The following table lists some of the materials and methods used in the cited studies.

Study	“Empty” Vector	Promoter/enhancer*	Cell type/tissue	“Empty” vector introduction
Cherington 1988	pZIP-neo	MMLV	3T3-F442A preadipocytes	Infection with retrovirus
Higgins 1996	HSV-neo	HSV	3T3-L1 preadipocytes	Calcium phosphate precipitate transfection
	pZIP-neo	MMLV	3T3-L1 preadipocytes	Calcium phosphate precipitate transfection
Awazu 1998	pSV-neo	SV40	HuH-7 human hepatoma cells	Lipofection transfection
Hu 2001	pcDNA3	CMV	Skin tumors	Injection into skin
Choi 2001	pcDNA3	CMV	human multiple myeloma (MM)-derived cells	Lipofectamine Plus transfection

* MMLV- Moloney Murine Leukemia Virus, SV40- Simian Virus No 40, CMV- Cytomegalovirus, HSV- Murine Harvey Sarcoma Virus

Although the cited studies used different materials and methods, such as different plasmids, different transfection methods, different cell types, and different organisms, the observations, in all studies, are consistent with the predicted effect of microcompetition.

The argument for large sample sizes, randomization, independent verification by different laboratories, etc, is to even out specific peculiarities inherent in any single measurement. The same result under dissimilar conditions is considered reliable. Since the effect of the “empty” vector on cell function was observed under a variety of conditions using dissimilar materials and methods, the effect is, most likely, not an artifact of any specific study, and therefore, reliable. The added reliability increases the level of confidence in the validity of the proposed new concept.

One of the most powerful instruments in the scientific tool bag is the paradigm, the mental model that represents reality⁵⁹. However, a paradigm is also a filter on perception. The above studies illustrate the blinding power of a paradigm. The current view holds that viral proteins are the sole mediators of viral effects on the host cell. Such proteins include, for example, the papillomavirus type 16 E6 and E7 oncoproteins, SV40 large T antigen, Epstein-Barr virus BRLF1 protein, and adenovirus E1A. Following the

“protein-dependent” assumption, the standard plasmid, which did not express the protein of interest, was called “empty,”³ and was used in preparing control cells. As expected, these cells were never compared with the other controls, the non-transfected cells. The viral “protein-dependent” view has such a strong hold on the scientists’ mind that even when a “protein-independent” effect on cell function presented itself in the laboratory, the effect was ignored.

The potential of a paradigm to bias perception should be considered when deciding the faith of future research on the proposed relation between latent viral infections and disease. A latent viral infection produces no protein, or almost no protein. If the few proteins expressed by the virus are harmless, a supporter of the “protein-dependent” view will conclude that the latent infection is harmless, and will refrain from advancing research on the matter. In contrast, a proponent of the “protein-independent” view will conclude that such an infection might be associated with disease, and will actively explore the issue. The objective of the report is to provide enough stimulation for further consideration of microcompetition, the “protein-independent” approach, and therefore, further consideration of the proposed association between latent viral infections and disease.

IV. An open question

The manuscript reports the discovery of a new biological mechanism and shows that disruption of this mechanism results in reduced cell differentiation and excessive cell proliferation. Consider the discovery of the double helix. According to Watson and Crick (1953⁶⁰): “It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.” The significance of the discovery lies not in the description of the molecular structure of DNA, but in the hinted mechanism for human inheritance.

When is the discovery of a new mechanism exciting? When the new mechanism can be used to answer important questions that resist conventional means. In the case of the double helix, inheritance was considered, at the time, one of the most intriguing and difficult questions in biology. The following sections present one of today’s important questions: Why is transcription of many wild-type genes reduced, or elevated, in cancer?, and proposes an answer based on the newly discovered mechanism of microcompetition.

A. The question

Many studies report reduced transcription of the BRCA1 gene in a majority of sporadic breast and ovarian tumors (Russell 2000⁶¹, Rio 1999⁶², Rice 1998⁶³, Magdinier 1998⁶⁴, Ozcelik 1998⁶⁵, Thompson 1995⁶⁶). For instance, Magdinier, *et al.*, report a statistically

³ Why did the authors choose the word “empty?” Note that the word empty, like the number zero, has two meanings: a relative and an absolute one. In relative terms, empty means less than full. So one answer might be that the standard plasmid is “emptier” compared to the “fuller” test gene plasmid. However, empty also means “nothing,” and therefore, another reason for the choice might be that the word “empty” supposed to indicate “no effect.” Since the “empty” plasmid had a significant effect on cell function, we chose to mark the word empty with quotation marks throughout the report.

significant decrease in BRCA1 mRNA in 94% of patients tested (Magdinier 1999, *ibid*, Fig. 2), Rio, *et al.*, (1999, *ibid*) report a decrease in BRCA1 mRNA in 100% of the six cell lines tested, and Russell, *et al.*, (Rio 2000, *ibid*) report a decrease in BRCA1 protein expression in 90% of the tested epithelial ovarian tumors. However, the reason for the decreased transcription is unknown. The two classical hypotheses, somatic mutations and promoter hypermethylation, were not confirmed. Somatic mutations of the BRCA1 gene are rare in sporadic breast and ovarian tumors (Russell 2000 (*ibid*), Rio 1999 (*ibid*), Futreal 1994⁶⁷, Merajver 1995⁶⁸), and hypermethylation of the BRCA1 promoter was demonstrated in only a small percentage of sporadic breast cancer samples (Cateau 1999⁶⁹, Magdinier 1998 (*ibid*), Rice 1998 (*ibid*), Dobrovic 1997⁷⁰). The majority of breast and ovarian tumors show neither somatic mutations nor BRCA1 promoter hypermethylation.

A similar pattern is observed with the Fas gene. Many studies detected a progressive decrease in Fas expression in many cancers (see Keane 1996⁷¹ for breast carcinomas, Gratas 1998⁷² for esophageal carcinomas, Strand 1996⁷³ for hepatocellular carcinomas, Moller 1994⁷⁴ for colon carcinomas, and Leithauser 1993⁷⁵ for lung carcinomas). The decrease in Fas expression resulted from reduced transcription. For instance, Das 2000⁷⁶ shows reduced Fas transcription in ovarian, cervical and endometrial carcinoma tissues, and in four ovarian and three cervical carcinoma cell lines. Butler 1998⁷⁷ demonstrates reduced Fas transcription in colon tumors, and Keane 1996 (*ibid*) shows reduced Fas mRNA levels in six out of seven breast cancer cell lines. As with BRCA1, the cause of the decrease in Fas transcription is unknown. The two classical hypotheses, somatic mutations and promoter hypermethylation, also fail in the case of the Fas gene. Allelic loss or somatic mutations of the Fas gene are rare (Bertoni 2000⁷⁸, Lee 1999A⁷⁹, Lee 1999B⁸⁰, Shin 1999⁸¹, Butler 1998, *ibid*), and no hypermethylation was observed in the Fas promoter (Butler 2000⁸²). The majority of carcinomas show no somatic mutations or Fas promoter methylation.

So why is transcription of BRCA1 and Fas genes reduced in cancer?

B. The logical reasoning

1. Step 1: A GABP virus disrupts transcription of BRCA1 and Fas genes

According to microcompetition (see details above):

$$\uparrow[\text{N-box}_v] \rightarrow \downarrow[\text{mRNA}_g], \text{ where } g = \text{BRCA1 or Fas}$$

2. Step 2: Disrupted transcription of BRCA1 and Fas genes leads to cancer

A decrease in BRCA1 mRNA results in an accelerated rate of cell proliferation, anchorage independent growth and tumorigenicity (Thompson 1995 (*ibid*), Fig 6, Fig 4a,c, Rao 1996⁸³, Fig 4). Retroviral transfer of a wild-type BRCA1 gene to breast and ovarian cancer cell lines inhibited growth *in vitro* (Hold 1996⁸⁴). A phase I clinical study

with gene transfer of BRCA1 to 12 patients with extensive metastatic cancer showed stable disease for 4-16 weeks in eight patients, tumor reduction in three patients and radiographic shrinkage of measurable disease in one patient (Tait 1997⁸⁵). Moreover, the majority of familial breast cancer and ovarian cancer cases result from germline mutations in the BRCA1 gene.

Note that the retinoblastoma susceptibility (Rb) gene, another cell cycle suppressor, is also a GABP stimulated gene (Sowa 1997⁸⁶)

Cell population is determined by balancing cell growth and cell death. Programmed cell death, or apoptosis, is the final step in a series of morphological and biochemical events. Fas antigen is a 48-kDA cell surface receptor homologous to the tumor necrosis factor (TNF) family of transmembrane proteins. Fas ligation by the Fas ligand, or by antibodies, triggers rapid cell apoptosis. The Fas induced apoptosis was initially identified in the immune system. Ligation of Fas induced apoptosis in activated T cells, B cells, and natural killer cells. In addition, Fas was identified in many epithelial cells. Although the role of Fas in non-lymphoid tissues is not completely understood, maintenance of normal cell turnover and removal of potentially oncogenic cells have been suggested. Consider, for example, the epithelial layer of colonic mucosa. These cells show a rapid rate of cell turnover and high expression of Fas. It is conceivable that the high rate of colonocyte removal is Fas induced. Moreover, germline mutations in the Fas gene are associated with spontaneous development of plasmacytoid tumor in *lpr* mice (Davidson 1998⁸⁷), and neoplasms in two autoimmune lymphoproliferative syndrome (ALPS) patients (Drappa 1996⁸⁸).

The association between the reduced transcription of the BRCA1, Fas and Rb genes and cell proliferation can be presented symbolically as follows ([Cell] denotes cell number):

$$\downarrow[\text{mRNA}_g] \rightarrow \uparrow[\text{Cell}], \text{ where } g = \text{BRCA1 or Fas}$$

3. Step 3: A latent infection with a GABP virus leads to cancer

The logical principle of transitive deduction can be defined as follows:

IF (A → B) **AND** (B → C)
THEN (A → C)

If A leads to B, and B leads to C, then A leads to C. The principle of transitive deduction can be extended to any number of steps.

Transitive deduction, also called in logical literature transitive entailment, or cut (the name is associated with the “cut” of the intermediate B), is a fundamental principle of logics. Consider Gabbay (1994⁸⁹): “Cut is a very basic rule in traditional logical systems and can be found in one form or another in each one of them.” Note that in *Elements of Biology*, (1965⁹⁰), Weisz stated: “Deductive logic is used extensively by scientists to obtain predictions from hypotheses. ... Most scientists are so accustomed

to deductive reasoning that formal construction of ‘if...then...’ statements is unnecessary in setting up experiments.” In logical literature the above form of transitive deduction is called unitary cut.

Apply transitive deduction to step 1 and 2 above (g = BRCA1 or Fas).

IF $\uparrow[\text{N-box}_v] \rightarrow \downarrow[\text{mRNA}_g]$ (Step 1), **AND** $\downarrow[\text{mRNA}_g] \rightarrow \uparrow[\text{Cell}]$ (Step 2)
THEN $\uparrow[\text{N-box}_v] \rightarrow \uparrow[\text{Cell}]$

An increase in the copy number of a viral N-box in a cell results in cell proliferation. Note that this is a “protein-independent” effect. The effect is independent of viral proteins, and, therefore, occurs in all infections with a GABP virus, and during all phases of the virus life cycle, including the persistent and latent phase.

C. The answer

A latent infection with a GABP virus decreases the availability of GABP•p300/cbp to the BRCA1 and Fas promoters. Since GABP•p300/cbp transactivates both genes, microcompetition with the latent virus decreases BRCA1 and Fas transcription. The decrease in BRCA1 and Fas transcription, and other GABP regulated genes, such as Rb, results in excessive cell proliferation. Therefore, a study that measures BRCA1 and Fas expression in cancer tissues is expected to find reduced transcription and reduced mRNA concentration.

D. Viral genomes in tumors

Many studies report the detection of viral genomes in human tumors. The following table summarizes some of these observations.

Virus	Cancer
Epstein-Bar virus (EBV)	Burkitt’s lymphoma (BL)
	Nasopharyngeal carcinoma (NPC)
	Hodgkin’s disease
	Polymorphic B cell lymphomas
SV40	B-cell lymphoproliferation in immunosuppressed individuals
	Brain tumors
Human T cell lymphotropic virus-I (HTLV-I)	Osteosarcomas
	Adult T-cell leukemia
Human papilloma virus (HPV)	Anogenital cancers
	Skin cancers
	Oral cancers
Hepatitis B virus (HBV)	Hepatocellular carcinoma
Hepatitis C virus (HCV)	Hepatocellular carcinoma
Human herpes virus 8 (HHV8, KSHV)	Kaposi’s sarcoma
	Body cavity lymphoma

See also recent reviews on human tumor viruses by Butel 2000⁹¹, zur Hausen 1999⁹², Hoppe-Seyler 1999⁹³. On EBV and breast cancer see Bonnet 1999⁹⁴, Labrecque 1995⁹⁵, and the editorial by Magrath and Bhatia 1999⁹⁶.

EBV, SV40 and HTLV-I are GABP viruses. The repeated detection of GABP viral DNA in many tumors supports the suggested relation between GABP viruses and cancer.

An interesting observation in some of these studies is the detection of viral DNA with no expression of viral proteins. Consider, for example, the studies on EBV in breast cancer, which report undetectable EBER expression in many cases positive for EBV DNA by *in situ* PCR (Bonnet 1999 and Labrecque 1995 above, see also discussion on this observation and more examples in the editorial by Magrath and Bhatia 1999, referenced above). Moreover, in many studies, the detected viral DNA was replication defective. These observations are inconsistent with the current “protein-dependent” view, and consistent with microcompetition, the proposed “protein-independent” approach.

¹ Watanabe H, Imai T, Sharp PA, Handa H. Identification of two transcription factors that bind to specific elements in the promoter of the adenovirus early-region 4. *Mol Cell Biol* 1988 8(3):1290-300. The transcription factor binds to the promoter of the adenovirus early-region 4 (E4). Hence the name E4 transcription factor 1.

² Atlas E, Stramwasser M, Whiskin K, Mueller CR. GA-binding protein alpha/beta is a critical regulator of the BRCA1 promoter. *Oncogene* 2000 Apr 6;19(15):1933-40.

³ Li XR, Chong AS, Wu J, Roebuck KA, Kumar A, Parrillo JE, Rapp UR, Kimberly RP, Williams JW, Xu X. Transcriptional regulation of Fas gene expression by GA-binding protein and AP-1 in T cell antigen receptor.CD3 complex-stimulated T cells. *J Biol Chem*. 1999 Dec 3;274(49):35203-10.

⁴ Bannert R, Avots A, Baier M, Serfling E, Kurth R. GA-binding protein factors, in concert with the coactivator CREB binding protein/p300, control the induction of the interleukin 16 promoter in T lymphocytes. *Proc. Natl. Acad. Sci. USA* 1999 96:1541-1546.

⁵ Avots A, Hoffmeyer A, Flory E, Cimanis A, Rapp UR, Serfling E. GABP factors bind to a distal interleukin 2 (IL-2) enhancer and contribute to c-Raf-mediated increase in IL-2 induction. *Molecular and Cellular Biology* 1997 17(8):4381-4389.

⁶ Lin JX, Bhat NK, John S, Queale WS, Leonard WJ. Characterization of the human interleukin-2 receptor beta-chain gene promoter: regulation of promoter activity by ets gene products. *Mol Cell Biol*. 1993 Oct;13(10):6201-10.

⁷ Markiewicz S, Bosselut R, Le Deist F, de Villartay JP, Hivroz C, Ghysdael J, Fischer A, de Saint Basile G. Tissue-specific activity of the gammac chain gene promoter depends upon an Ets binding site and is regulated by GA-binding protein. *J Biol Chem*. 1996 Jun 21;271(25):14849-55.

⁸ Smith MF Jr, Carl VS, Lodie T, Fenton MJ. Secretory interleukin-1 receptor antagonist gene expression requires both a PU.1 and a novel composite NF-kappaB/PU.1/ GA-binding protein binding site. *J Biol Chem*. 1998 Sep 11;273(37):24272-9.

⁹ Sowa Y, Shiio Y, Fujita T, Matsumoto T, Okuyama Y, Kato D, Inoue J, Sawada J, Goto M, Watanabe H, Handa H, Sakai T. Retinoblastoma binding factor 1 site in the core promoter region of the human RB gene is activated by hGABP/E4TF1. *Cancer Res*. 1997 Aug 1;57(15):3145-8.

¹⁰ Kamura T, Handa H, Hamasaki N, Kitajima S. Characterization of the human thrombopoietin gene promoter. A possible role of an Ets transcription factor, E4TF1/GABP. *J Biol Chem*. 1997 Apr 25;272(17):11361-8.

¹¹ Wang K, Bohren KM, Gabbay KH. Characterization of the human aldose reductase gene promoter. *J Biol Chem*. 1993 Jul 25;268(21):16052-8.

-
- ¹² Nuchprayoon I, Shang J, Simkevich CP, Luo M, Rosmarin AG, Friedman AD. An enhancer located between the neutrophil elastase and proteinase 3 promoters is activated by Sp1 and an Ets factor. *J Biol Chem*. 1999 Jan 8;274(2):1085-91.
- ¹³ Nuchprayoon I, Simkevich CP, Luo M, Friedman AD, Rosmarin AG. GABP cooperates with c-Myb and C/EBP to activate the neutrophil elastase promoter. *Blood*. 1997 Jun 15;89(12):4546-54.
- ¹⁴ Sadasivan E, Cedeno MM, Rothenberg SP. Characterization of the gene encoding a folate-binding protein expressed in human placenta. Identification of promoter activity in a G-rich SP1 site linked with the tandemly repeated GGAAG motif for the ets encoded GA-binding protein. *J Biol Chem*. 1994 Feb 18;269(7):4725-35.
- ¹⁵ Basu A, Park K, Atchison ML, Carter RS, Avadhani NG. Identification of a transcriptional initiator element in the cytochrome c oxidase subunit Vb promoter which binds to transcription factors NF-E1 (YY-1, delta) and Sp1. *J Biol Chem*. 1993 Feb 25;268(6):4188-96.
- ¹⁶ Sucharov C, Basu A, Carter RS, Avadhani NG. A novel transcriptional initiator activity of the GABP factor binding ets sequence repeat from the murine cytochrome c oxidase Vb gene. *Gene Expr*. 1995;5(2):93-111.
- ¹⁷ Carter RS, Avadhani NG. Cooperative binding of GA-binding protein transcription factors to duplicated transcription initiation region repeats of the cytochrome c oxidase subunit IV gene. *J Biol Chem*. 1994 Feb 11;269(6):4381-7.
- ¹⁸ Carter RS, Bhat NK, Basu A, Avadhani NG. The basal promoter elements of murine cytochrome c oxidase subunit IV gene consist of tandemly duplicated ets motifs that bind to GABP-related transcription factors. *J Biol Chem*. 1992 Nov 15;267(32):23418-26.
- ¹⁹ Virbasius JV, Scarpulla RC. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc Natl Acad Sci U S A*. 1994 Feb 15;91(4):1309-13.
- ²⁰ Villena JA, Vinas O, Mampel T, Iglesias R, Giralt M, Villarroya F. Regulation of mitochondrial biogenesis in brown adipose tissue: nuclear respiratory factor-2/GA-binding protein is responsible for the transcriptional regulation of the gene for the mitochondrial ATP synthase beta subunit. *Biochem J*. 1998 Apr 1;331 (Pt 1):121-7.
- ²¹ Ouyang L, Jacob KK, Stanley FM. GABP mediates insulin-increased prolactin gene transcription. *J Biol Chem*. 1996 May 3;271(18):10425-8.
- ²² Hoare S, Copland JA, Wood TG, Jeng YJ, Izbán MG, Soloff MS. Identification of a GABP alpha/beta binding site involved in the induction of oxytocin receptor gene expression in human breast cells, potentiation by c-Fos/c-Jun. *Endocrinology*. 1999 May;140(5):2268-79.
- ²³ Asano M, Murakami Y, Furukawa K, Yamaguchi-Iwai Y, Stake M, Ito Y. A Poliovirus Enhancers Binding Protein, PEBP5, Responsive to 12-O-Tetradecanoylphorbol-13-Acetate but Distinct From AP-1. *Journal of Virology* 1990 64(12):5927-5938.
- ²⁴ Higashino F, Yoshida K, Fujinaga Y, Kamio K, Fujinaga K. Isolation fo a cDNA Encoding the Adenovirus E1A Enhancer Binding Protein: A New Human Member of the ets Oncogene Family. *Nucleic Acids Research* 1993 21(3):547-553.
- ²⁵ Laimins LA, Tschlis P, Khoury G. Multiple Enhancer Domains in the 3' Terminus of the Prague Strain of Rous Sarcoma Virus. *Nucleic Acids Research* 1984 12(16):6427-6442.
- ²⁶ LaMarco KL, McKnight SL. Purification of a set of cellular polypeptides that bind to the purine-rich cis-regulatory element of herpes simplex virus immediate early genes. *Genes Dev* 1989 3(9):1372-83.
- ²⁷ Douville P, Hagmann M, Georgiev O, Schaffner W. Positive and negative regulation at the herpes simplex virus ICP4 and ICP0 TAATGARAT motifs. *Virology*. 1995 Feb 20;207(1):107-16.
- ²⁸ Boshart M, Weber F, Jahn G, Dorsch-Hasler K, Fleckenstein B, Schaffner W. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 1985 Jun;41(2):521-30.
- ²⁹ Gunther CV, Graves BJ. Identification of ETS domain proteins in murine T lymphocytes that interact with the Moloney murine leukemia virus enhancer. *Mol Cell Biol* 1994 14(11): 7569-80
- ³⁰ Flory E, Hoffmeyer A, Smola U, Rapp UR, Bruder JT. Raf-1 kinase targets GA-binding protein in transcriptional regulation of the human immunodeficiency virus type 1 promoter. *J Virol* 1996 Apr;70(4):2260-8.

-
- ³¹ Rawlins DR, Milman G, Hayward SD, Hayward GS. Sequence-specific DNA binding of the Epstein-Barr virus nuclear antigen (EBNA-1) to clustered sites in the plasmid maintenance region. *Cell* 1985 Oct;42(3):859-68.
- ³² Mauclere P, Mahieux R, Garcia-Calleja JM, Salla R, Tekaiia F, Millan J, De The G, Gessain A. A new HTLV-II subtype A isolate in an HIV-1 infected prostitute from Cameroon, Central Africa. *AIDS Res Hum Retroviruses*. 1995 Aug;11(8):989-93.
- ³³ Kornfeld H, Riedel N, Viglianti GA, Hirsch V, Mullins JI. Cloning of HTLV-4 and its relation to simian and human immunodeficiency viruses. *Nature* 1987 326(6113):610-613.
- ³⁴ Bruder JT, Hearing P. Cooperative binding of EF-1A to the E1A enhancer region mediates synergistic effects on E1A transcription during adenovirus infection. *J Virol*. 1991 Sep;65(9):5084-7.
- ³⁵ Bruder JT, Hearing P. Nuclear factor EF-1A binds to the adenovirus E1A core enhancer element and to other transcriptional control regions. *Mol Cell Biol*. 1989 Nov;9(11):5143-53.
- ³⁶ Ostapchuk P, Diffley JF, Bruder JT, Stillman B, Levine AJ, Hearing P. Interaction of a nuclear factor with the polyomavirus enhancer region. *Proc Natl Acad Sci U S A*. 1986 Nov;83(22):8550-4.
- ³⁷ Scholer HR, Gruss P. Specific interaction between enhancer-containing molecules and cellular components. *Cell*. 1984 Feb;36(2):403-11.
- ³⁸ Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, Lin SC, Heyman RA, Rose DW, Glass CK, Rosenfeld MG. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell*. 1996 May 3;85(3):403-14.
- ³⁹ Horvai AE, Xu L, Korzus E, Brard G, Kalafus D, Mullen TM, Rose DW, Rosenfeld MG, Glass CK. Nuclear integration of JAK/STAT and Ras/AP-1 signaling by CBP and p300. *Proc Natl Acad Sci U S A*. 1997 Feb 18;94(4):1074-9.
- ⁴⁰ Hottiger MO, Felzien LK, Nabel GJ. Modulation of cytokine-induced HIV gene expression by competitive binding of transcription factors to the coactivator p300. *EMBO J*. 1998 Jun 1;17(11):3124-34.
- ⁴¹ Pise-Masison CA, Mahieux R, Radonovich M, Jiang H, Brady JN. Human T-lymphotropic virus type I Tax protein utilizes distinct pathways for p53 inhibition that are cell type-dependent. *J Biol Chem*. 2001 Jan 5;276(1):200-5.
- ⁴² Banas B, Eberle J, Banas B, Schlondorff D, Luckow B. Modulation of HIV-1 enhancer activity and virus production by cAMP. *FEBS Lett*. 2001 Dec 7;509(2):207-12.
- ⁴³ Wang C, Fu M, D'Amico M, Albanese C, Zhou JN, Brownlee M, Lisanti MP, Chatterjee VK, Lazar MA, Pestell RG. Inhibition of cellular proliferation through I κ B kinase-independent and peroxisome proliferator-activated receptor gamma-dependent repression of cyclin D1. *Mol Cell Biol*. 2001 May;21(9):3057-70.
- ⁴⁴ Ernst P, Wang J, Huang M, Goodman RH, Korsmeyer SJ. MLL and CREB bind cooperatively to the nuclear coactivator CREB-binding protein. *Mol Cell Biol*. 2001 Apr;21(7):2249-58.
- ⁴⁵ Yuan W, Varga J. Transforming growth factor-beta repression of matrix metalloproteinase-1 in dermal fibroblasts involves Smad3. *J Biol Chem*. 2001 Oct 19;276(42):38502-10.
- ⁴⁶ Ghosh AK, Yuan W, Mori Y, Chen S, Varga J. Antagonistic regulation of type I collagen gene expression by interferon-gamma and transforming growth factor-beta. Integration at the level of p300/CBP transcriptional coactivators. *J Biol Chem*. 2001 Apr 6;276(14):11041-8.
- ⁴⁷ Li M, Pascual G, Glass CK. Peroxisome proliferator-activated receptor gamma-dependent repression of the inducible nitric oxide synthase gene. *Mol Cell Biol*. 2000 Jul;20(13):4699-707.
- ⁴⁸ Nagarajan RP, Chen F, Li W, Vig E, Harrington MA, Nakshatri H, Chen Y. Repression of transforming-growth-factor-beta-mediated transcription by nuclear factor kappaB. *Biochem J*. 2000 Jun 15;348 Pt 3:591-6.
- ⁴⁹ Speir E, Yu ZX, Takeda K, Ferrans VJ, Cannon RO 3rd. Competition for p300 regulates transcription by estrogen receptors and nuclear factor-kappaB in human coronary smooth muscle cells. *Circ Res*. 2000 Nov 24;87(11):1006-11.
- ⁵⁰ Chen YH, Ramos KS. A CCAAT/enhancer-binding protein site within antioxidant/electrophile response element along with CREB-binding protein participate in the negative regulation of rat GST-Ya gene in vascular smooth muscle cells. *J Biol Chem*. 2000 Sep 1;275(35):27366-76.
- ⁵¹ Werner F, Jain MK, Feinberg MW, Sibinga NE, Pellacani A, Wiesel P, Chin MT, Topper JN, Perrella MA, Lee ME. Transforming growth factor-beta 1 inhibition of macrophage activation is mediated via Smad3. *J Biol Chem*. 2000 Nov 24;275(47):36653-8.

-
- ⁵² Cherington V, Brown M, Paucha E, St Louis J, Spiegelman BM, Roberts TM. Separation of simian virus 40 large-T-antigen-transforming and origin-binding functions from the ability to block differentiation. *Mol Cell Biol.* 1988 Mar;8(3):1380-4.
- ⁵³ Cepko CL, Roberts BE, Mulligan RC. Construction and applications of a highly transmissible murine retrovirus shuttle vector. *Cell.* 1984 Jul;37(3):1053-62.
- ⁵⁴ Higgins C, Chatterjee S, Cherington V. The block of adipocyte differentiation by a C-terminally truncated, but not by full-length, simian virus 40 large tumor antigen is dependent on an intact retinoblastoma susceptibility protein family binding domain. *J Virol.* 1996 Feb;70(2):745-52.
- ⁵⁵ Armelin HA, Armelin MC, Kelly K, Stewart T, Leder P, Cochran BH, Stiles CD. Functional role for c-myc in mitogenic response to platelet-derived growth factor. *Nature.* 1984 Aug 23-29;310(5979):655-60.
- ⁵⁶ Awazu S, Nakata K, Hida D, Sakamoto T, Nagata K, Ishii N, Kanematsu T. Stable transfection of retinoblastoma gene promotes contact inhibition of cell growth and hepatocyte nuclear factor-1-mediated transcription in human hepatoma cells. *Biochem Biophys Res Commun.* 1998 Nov 9;252(1):269-73.
- ⁵⁷ Choi SJ, Oba Y, Gazitt Y, Alsina M, Cruz J, Anderson J, Roodman GD. Antisense inhibition of macrophage inflammatory protein 1-alpha blocks bone destruction in a model of myeloma bone disease. *J Clin Invest.* 2001 Dec;108(12):1833-41.
- ⁵⁸ Hu Z, Garen A. Targeting tissue factor on tumor vascular endothelial cells and tumor cells for immunotherapy in mouse models of prostatic cancer. *Proc Natl Acad Sci U S A.* 2001 Oct 9;98(21):12180-5.
- ⁵⁹ Kuhn ST. *The Structure of Scientific Revolution.* The University of Chicago Press. (1962).
- ⁶⁰ Watson JD, Crick FHC. Molecular Structure of Nucleic Acid. *Nature,* 1953, 737-738.
- ⁶¹ Russell PA, Pharoah PD, De Foy K, Ramus SJ, Symmonds I, Wilson A, Scott I, Ponder BA, Gayther SA. Frequent loss of BRCA1 mRNA and protein expression in sporadic ovarian cancers. *Int J Cancer.* 2000 Aug;87(3):317-321.
- ⁶² Rio PG, Maurizis JC, Peffault de Latour M, Bignon YJ, Bernard-Gallon DJ. Quantification of BRCA1 protein in sporadic breast carcinoma with or without loss of heterozygosity of the BRCA1 gene. *Int J Cancer* 1999 Mar 15;80(6):823-6.
- ⁶³ Rice JC, Massey-Brown KS, Futscher BW. Aberrant methylation of the BRCA1 CpG island promoter is associated with decreased BRCA1 mRNA in sporadic breast cancer cells. *Oncogene.* 1998 Oct 8;17(14):1807-12.
- ⁶⁴ Magdinier F, Ribieras S, Lenoir GM, Frappart L, Dante R. Down-regulation of BRCA1 in human sporadic breast cancer; analysis of DNA methylation patterns of the putative promoter region. *Oncogene.* 1998 Dec 17;17(24):3169-76.
- ⁶⁵ Ozelik H, To MD, Couture J, Bull SB, Andrulis IL. Preferential allelic expression can lead to reduced expression of BRCA1 in sporadic breast cancers. *Int J Cancer.* 1998 Jul 3;77(1):1-6.
- ⁶⁶ Thompson ME, Jensen RA, Obermiller PS, Page DL, Holt JT. Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. *Nat Genet* 1995 Apr;9(4):444-50.
- ⁶⁷ Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, et al. BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 1994 Oct 7;266(5182):120-2.
- ⁶⁸ Merajver SD, Pham TM, Caduff RF, Chen M, Poy EL, Cooney KA, Weber BL, Collins FS, Johnston C, Frank TS. Somatic mutations in the BRCA1 gene in sporadic ovarian tumours. *Nat Genet.* 1995 Apr;9(4):439-43.
- ⁶⁹ Catteau A, Harris WH, Xu CF, Solomon E. Methylation of the BRCA1 promoter region in sporadic breast and ovarian cancer: correlation with disease characteristics. *Oncogene.* 1999 Mar 18;18(11):1957-65.
- ⁷⁰ Dobrovic A, Simpfendorfer D. Methylation of the BRCA1 gene in sporadic breast cancer. *Cancer Res.* 1997 Aug 15;57(16):3347-50.
- ⁷¹ Keane MM, Ettenberg SA, Lowrey GA, Russell EK, Lipkowitz S. Fas expression and function in normal and malignant breast cell lines. *Cancer Res.* 1996 Oct 15;56(20):4791-8.
- ⁷² Gratas C, Tohma Y, Barnas C, Taniere P, Hainaut P, Ohgaki H. Up-regulation of Fas (APO-1/CD95) ligand and down-regulation of Fas expression in human esophageal cancer. *Cancer Res.* 1998 May 15;58(10):2057-62.

-
- ⁷³ Strand S, Hofmann WJ, Hug H, Muller M, Otto G, Strand D, Mariani SM, Stremmel W, Krammer PH, Galle PR. Lymphocyte apoptosis induced by CD95 (APO-1/Fas) ligand-expressing tumor cells--a mechanism of immune evasion? *Nat Med.* 1996 Dec;2(12):1361-6.
- ⁷⁴ Moller P, Koretz K, Leithauser F, Bruderlein S, Henne C, Quentmeier A, Krammer PH. Expression of APO-1 (CD95), a member of the NGF/TNF receptor superfamily, in normal and neoplastic colon epithelium. *Int J Cancer.* 1994 May 1;57(3):371-7.
- ⁷⁵ Leithauser F, Dhein J, Mechttersheimer G, Koretz K, Bruderlein S, Henne C, Schmidt A, Debatin KM, Krammer PH, Moller P. Constitutive and induced expression of APO-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily, in normal and neoplastic cells. *Lab Invest.* 1993 Oct;69(4):415-29.
- ⁷⁶ Das H, Koizumi T, Sugimoto T, Chakraborty S, Ichimura T, Hasegawa K, Nishimura R. Quantitation of Fas and Fas ligand gene expression in human ovarian, cervical and endometrial carcinomas using real-time quantitative RT-PCR. *Br J Cancer.* 2000 May;82(10):1682-8.
- ⁷⁷ Butler LM, Hewett PJ, Butler WJ, Cowled PA. Down-regulation of Fas gene expression in colon cancer is not a result of allelic loss or gene rearrangement. *Br J Cancer.* 1998 May;77(9):1454-9.
- ⁷⁸ Bertoni F, Conconi A, Carobbio S, Realini C, Codegoni AM, Zucca E, Cavalli F. Analysis of Fas/CD95 gene somatic mutations in ovarian cancer cell lines. *Int J Cancer.* 2000 May 1;86(3):450.
- ⁷⁹ Lee SH, Shin MS, Park WS, Kim SY, Kim HS, Han JY, Park GS, Dong SM, Pi JH, Kim CS, Kim SH, Lee JY, Yoo NJ. Alterations of Fas (Apo-1/CD95) gene in non-small cell lung cancer. *Oncogene.* 1999 Jun 24;18(25):3754-60.
- ⁸⁰ Lee SH, Shin MS, Park WS, Kim SY, Dong SM, Pi JH, Lee HK, Kim HS, Jang JJ, Kim CS, Kim SH, Lee JY, Yoo NJ. Alterations of Fas (APO-1/CD95) gene in transitional cell carcinomas of urinary bladder. *Cancer Res* 1999 Jul 1;59(13):3068-72.
- ⁸¹ Shin MS, Park WS, Kim SY, Kim HS, Kang SJ, Song KY, Park JY, Dong SM, Pi JH, Oh RR, Lee JY, Yoo NJ, Lee SH. Alterations of Fas (Apo-1/CD95) gene in cutaneous malignant melanoma. *Am J Pathol* 1999 Jun;154(6):1785-91.
- ⁸² Butler LM, Dobrovic A, Bianco T, Cowled PA. Promoter region methylation does not account for the frequent loss of expression of the Fas gene in colorectal carcinoma. *Br J Cancer.* 2000 Jan;82(1):131-5.
- ⁸³ Rao VN, Shao N, Ahmad M, Reddy ES. Antisense RNA to the putative tumor suppressor gene BRCA1 transforms mouse fibroblasts. *Oncogene.* 1996 Feb 1;12(3):523-8.
- ⁸⁴ Holt JT, Thompson ME, Szabo C, Robinson-Benion C, Arteaga CL, King MC, Jensen RA. Growth retardation and tumour inhibition by BRCA1. *Nat Genet.* 1996 Mar;12(3):298-302.
- ⁸⁵ Tait DL, Obermiller PS, Redlin-Frazier S, Jensen RA, Welch P, Dann J, King MC, Johnson DH, Holt JT. A phase I trial of retroviral BRCA1sv gene therapy in ovarian cancer. *Clin Cancer Res.* 1997 Nov;3(11):1959-68.
- ⁸⁶ Sowa Y, Shii Y, Fujita T, Matsumoto T, Okuyama Y, Kato D, Inoue J, Sawada J, Goto M, Watanabe H, Handa H, Sakai T. Retinoblastoma binding factor 1 site in the core promoter region of the human RB gene is activated by hGABP/E4TF1. *Cancer Res.* 1997 Aug 1;57(15):3145-8.
- ⁸⁷ Davidson WF, Giese T, Fredrickson TN. Spontaneous development of plasmacytoid tumors in mice with defective Fas-Fas ligand interactions. *J Exp Med.* 1998 Jun 1;187(11):1825-38.
- ⁸⁸ Drappa J, Vaishnav AK, Sullivan KE, Chu JL, Elkon KB. Fas gene mutations in the Canale-Smith syndrome, an inherited lymphoproliferative disorder associated with autoimmunity. *N Engl J Med.* 1996 Nov 28;335(22):1643-9.
- ⁸⁹ Gabbay DM. What is a logical system. In Gabbay DM, editor, *What is a logical system?* Clarendon Press, Oxford, 1994. pp 179-216.
- ⁹⁰ Weisz P. *Elements of Biology* (New York: McGraw-Hill). 1965. p 8
- ⁹¹ Butel JS. *Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease.* Carcinogenesis. 2000 Mar;21(3):405-26.
- ⁹² zur Hausen H. Viruses in human cancers. *Eur J Cancer.* 1999 Aug;35(8):1174-81.
- ⁹³ Hoppe-Seyler F, Butz K. Human tumor viruses. *Anticancer Res.* 1999 Nov-Dec;19(6A):4747-58.
- ⁹⁴ Bonnet M, Guinebretiere JM, Kremmer E, Grunewald V, Benhamou E, Contesso G, Joab I. Detection of Epstein-Barr virus in invasive breast cancers. *J Natl Cancer Inst.* 1999 Aug 18;91(16):1376-81.
- ⁹⁵ Labrecque LG, Barnes DM, Fentiman IS, Griffin BE. Epstein-Barr virus in epithelial cell tumors: a breast cancer study. *Cancer Res.* 1995 Jan 1;55(1):39-45.

⁹⁶ Magrath I, Bhatia K. Breast cancer: a new Epstein-Barr virus-associated disease? J Natl Cancer Inst. 1999 Aug 18;91(16):1349-50.