A new method for the selection of protein interactions in mammalian cells

Eileen ROJO-NIERSBACH, Debra MORLEY, Stephanie HECK, and Norbert LEHMING¹

Max-Delbrück-Laboratorium in der Max-Planck-Gesellschaft, Carl-von-Linné-Weg 10, 50829 Köln, Germany

In the present study we present a new method that allows for the selection of protein interactions in mammalian cells. We have used this system to verify two interactions previously characterized *in vitro*. (1) The interaction between human TATA-binding protein 1 and nuclear factor κ B and (2) the association of *Homo sapiens* nuclear autoantigen SP100B with human heterochromatin protein 1 α , a protein implicated in chromatin remodelling. We observe for the first time that these interactions also occur *in vivo*. One protein was fused to the N-terminal half of ubiquitin, while the interacting partner was fused to the C-terminal half of ubiquitin, that was itself linked to guanine phosphoryltransferase 2 (gpt2) modified to begin with an arginine residue. Upon interaction of both proteins, ubiquitin is reconstituted, and its association with the Rgpt2 reporter is

subsequently cleaved off by ubiquitin-processing enzymes. The presence of arginine in the Rgpt2 gene product leads to the degradation of the product by the N-end rule pathway. In the human fibroblast cell line HT1080HPRT[−] (that is deficient in the enzyme for hypoxanthine-guanine phosphoribosyltransferase) cells in which interaction between both proteins of interest occurs can then be selected for by hypoxanthine/aminopterin/thymine medium and counterselected against by 6-thioguanine medium. This method provides a suitable alternative to the yeast two-hybrid system and is generally applicable.

Key words: *Homo sapiens* nuclear autoantigen 100B, human heterochromatin protein 1 α , human TATA-binding protein 1, nuclear factor κ B, transcription.

INTRODUCTION

Protein interactions are necessary for all biological processes. These interactions can be studied with purified proteins *in vitro*, but these studies must be validated for their biological significance inside the living cell. For example, the yeast two-hybrid assay can be used to functionally analyse protein interactions *in vivo*. This assay was first described for *Saccharomyces cerevisiae* [1,2] and has since been adapted to monitor protein interactions in tissue culture cells [3]. However, the assay is based on a transcriptional readout; as a consequence, the analysis of transcription factors is limited. For example, transcriptional repressors may interfere with the signal, while transcriptional activators may produce a signal in the absence of an interacting protein. Also, the interaction must take place inside the nucleus, thereby excluding, for example, the analysis of membrane proteins in their native environment. An alternative system based on cell proliferation has been described for *S. cerevisiae* [4], and this system has recently been adapted for mammalian cells [5]. This system allows transcription factors to be tested; however, membrane proteins cannot be studied. Additionally, it is only possible to monitor protein interactions, because a selection method has not yet been described.

The split-ubiquitin assay is an alternative to the standard yeast two-hybrid system [6]. Ubiquitin is split into N-terminal and C-terminal halves (N_{ub} and C_{ub}, respectively), and each half is fused to either protein of interest. If the two proteins interact inside the living cell, the two halves of ubiquitin are brought into close contact; hence, this complemented ubiquitin is recognized by the ubiquitin-specific proteases (UBPs). The fusion containing C_{ub} is cleaved, resulting in a protein product of reduced size. Since ubiquitin proteases are present in the cytosol and in the nucleus

[7,8], the assay is not limited to interactions reconstituted in the nucleus of the cell. The split-ubiquitin assay was first described for *S. cerevisiae*, and the analysis was performed with Western blots [9]. Recently, a selection in *S. cerevisiae* based on the split-ubiquitin assay has been published [10]. One protein is fused to N_{ub}, while a second protein is fused to C_{ub} followed by orotidine-5'-phosphate decarboxylase (ura3) with an arginine at position 1. As shown by the N-end rule [11], which correlates the *in vivo* half-life of a protein with the nature of its N-terminal residue, introduction of this arginine residue promotes the rapid degradation of the Rura3 gene product. If the two proteins of interest interact inside the living cell, ubiquitin is reconstituted and the C_{ub} fusion protein is cleaved from the Rura3 gene product. Upon its cleavage from the C_{ub} fusion protein, the Rura3 reporter is degraded; as a result, the yeast cells become phenotypically uracil-deficient. 5-Fluoroorotic acid (FOA) can be used to select for uracil-deficient *S. cerevisiae* cells; therefore, the protein interactions inside the living cells can be selected for (with uracil-depleted medium) and counterselected against (with medium containing FOA).

We utilize this system to verify protein–protein interactions implicated in the transcriptional regulation of human genes. Previously, it has been demonstrated that human TATA-binding protein 1 (hTBP1) interacts with the C-terminal activation domain (amino acids 305–551) of nuclear factor κ B (NF- κ B), known as p65C, *in vitro* [12], and that co-transfection of hTBP1 with NF- κ B augments transcription [13]. In *S. cerevisiae* it has recently been demonstrated that the occupancy of TBP on the TATA-box is increased in response to transcriptional activators [14,15]. Even though the presence of other factors, like transcription factor IIB, on active promoters is increased as well, the binding of TBP to the TATA-box is considered to be a crucial

Abbreviations used: C_{ub}, C-terminal half of ubiquitin; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; FOA, 5-fluoroorotic acid; gpt2, guanine phosphoryltransferase 2; HA, haemagglutinin; HAT, hypoxanthine/aminopterin/thymidine; hHP1 α , human heterochromatin protein 1 α ; HPRT, hypoxanthine-guanine phosphoribosyltransferase; hSP100B, *Homo sapiens* nuclear autoantigen 100B; hTBP1, human TATA-binding protein 1; N_{ub}, N-terminal half of ubiquitin; NF- κ B, nuclear factor κ B; p65C, C-terminal activation domain of NF- κ B; 6TG, 6-thioguanine; UBPs, ubiquitin-specific proteases; ura3, orotidine-5'-phosphate decarboxylase.

¹ To whom correspondence should be addressed (e-mail lehmig@mpiz-koeln.mpg.de).

step for transcriptional activation, and direct binding of TBP to activation domains could explain transcriptional activation by a simple recruitment model [16].

Likewise, it has been shown that *Homo sapiens* nuclear autoantigen (hSP100B), a splice variant of the nuclear dot protein hSP100A, binds to the repressing non-histone chromosomal protein, human heterochromatin protein 1 α (hHP1 α), *in vitro* [17]. hHP1 α , in the context of the Gal4 DNA-binding domain, has also been demonstrated to repress transcription of a promoter containing Gal4-DNA binding sites in mammalian cells, and its ability to repress depends on DNA binding [17]. This result suggests that the interaction between hSP100B and hHP1 α may be functionally relevant to the ability of hHP1 α to repress transcription.

In the present study we have developed a split-ubiquitin system that allows for the selection of protein interactions inside the living human cell. Using this system we demonstrate for the first time *in vivo* two previously characterized interactions, one between hTBP1 and human NF- κ B and the other between hSP100B and hHP1 α .

MATERIALS AND METHODS

Standard protocols [18] were used for all procedures, unless otherwise indicated.

Cell lines and *Escherichia coli* strains

The human fibroblast cell line HT1080HPRT[−], which is deficient in the enzyme for hypoxanthine-guanine phosphoribosyltransferase (HPRT) was obtained from Sandra Pellegrini (Department of Immunology, Institut Pasteur, Paris, France) [19]. Stable cell lines were made by lipofectamine transfections (Life Technologies, Eggenstein-Leopoldshafen, Germany) according to the manufacturer's protocol. Cell lines containing the C_{ub} fusion vectors were selected for by resistance to zeocin (Invitrogen, Leek, The Netherlands), while those containing the N_{ub} fusion vectors were selected for by resistance to G418 (PAA Laboratories, Cölbe, Germany).

Plasmids

The N_{ub} fusion vector was created by inserting a *Hind*III/*Eco*RI-restricted PCR fragment containing N_{ub} into pcDNA3 (Invitrogen), and the C_{ub} fusion vector was created by placing a *Xho*I/*Apa*I-cut PCR fragment containing a fusion of C_{ub} with guanine phosphoryltransferase 2 (gpt2), modified to begin with an arginine residue, into pcDNA3+zeocin (Invitrogen). PCR fragments containing the cDNAs of hTBP1 and p65C were cut with *Xho*I/*Xba*I and *Eco*RI/*Xho*I, respectively, and inserted into the two vectors to create the N_{ub}-hTBP1 and p65C-C_{ub}-Rgpt2 fusions. Likewise, cDNAs of hHP1 α and hSP100B, generated via PCR and cut with *Eco*RI/*Sa*I, were subcloned into the same vectors to create the respective N_{ub}-hHP1 α and hSP100B-C_{ub}-Rgpt2 fusions. The following primers were used: N_{ub}, 5'-GCCAAGCTTATGCAGATTTTCGTCAAGAC-3' and 5'-G-GCGAATTCAGCGTAATCTGGAACATC-3'; C_{ub}-Rgpt2, 5'-GCCCTCAGCGGATATCCCTCCAGATCAA-3' and 5'-G-CCGGGCCCTTAGCGACCGGAGATT-3'; hTBP1, 5'-GGC-CTCGAGTATGGATCAGAACAACAGCCT-3' and 5'-GG-CTCTAGATTACGTCTTCTGAATC-3'; p65C, 5'-GCCG-AATTCATGGTGACATATGAGACCTTCA-3' and 5'-CTC-GAGCTGATCTGACTCAGCAGGGC-3'; hHP1 α , 5'-GCC-GAATTCATGGGAAAGAAAACCAAGCG-3' and 5'-GCC-GTCGACGCGCTCTTTGCTGTTTCTTCTC-3'; hSP100B,

5'-GCCGAATTCATGGCAGGTGGGGGCGGCGAA-3' and 5'-GGCGTCGACTTTTCCAAAGTAGAATTGTTTT-3'.

Selections

HT1080HPRT[−] cells were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal calf serum (FCS). Zeocin (80 mg/l) was added to select for the C_{ub} fusions and G418 (400 mg/l) was added to select for the N_{ub} fusions. Single colonies were picked and several stable cell lines were analysed for each construct. Hypoxanthine/aminopterin/thymidine (HAT) medium was made according to the manufacturer's protocol (Roche, Mannheim, Germany), as well as 6-thioguanine (6TG) medium (15 mg/l) (Sigma, Deisenhofen, Germany).

Western-blots

Proteins were separated by SDS/PAGE and transferred to nitrocellulose membranes (Sartorius, Göttingen, Germany) by semi-dry blotting (Pharmacia, Freiburg, Germany). The membranes were probed with anti-haemagglutinin (HA) antibody (BabCO, Freiburg, Germany). Secondary horseradish peroxidase-coupled anti-mouse antibody (Bio-Rad, München, Germany) was used to detect the proteins with the help of the ECL⁺ system (Amersham International, Braunschweig, Germany).

RESULTS

The split-ubiquitin assay was originally developed for *S. cerevisiae*, utilizing the *ura3* gene for selection (absence of uracil) and counterselection (presence of FOA) of protein interactions inside the living cell. The readout in this assay configuration is based on proteolytic degradation and not on transcription; therefore, it can be used to analyse transcription factors, among others. In order to adapt this elegant system for mammalian cells we used the human fibroblast cell line HT1080HPRT[−] [19]. This cell line is deficient in the enzyme for HPRT, and thus is sensitive to medium containing HAT, but is resistant to medium containing 6TG. This deficiency can be complemented with the *E. coli* gene for gpt2. In order to test if Rgpt2 was a substrate of the N-end rule, we produced two stable cell lines: one expressing gpt2 as a fusion to the full-length ubiquitin with a glycine in position 1 after cleavage and one with an arginine in this position. Both fusions containing full-length ubiquitin were rapidly cleaved into ubiquitin and the corresponding gpt2 protein by the UBPs. If Rgpt2 was a good substrate for the N-end rule in mammalian cells, the HT1080HPRT[−] cells carrying ubiquitin-Rgpt2 should have been HAT-sensitive and 6TG-resistant, whereas the cells

Table 1 Rgpt2 is rapidly degraded in human fibroblast cells

The parental cell line HT1080HPRT[−] is sensitive to zeocin and HAT but resistant to 6TG. A stable cell line, created with the help of the zeocin marker, that expresses a fusion of ubiquitin to gpt2 with a glycine in position 1 (ubiquitin-Ggpt2) is resistant to zeocin and HAT but sensitive to 6TG. If the glycine is replaced by an arginine (ubiquitin-Rgpt2) the cell line becomes resistant to zeocin and 6TG but sensitive to HAT.

HT1080HPRT [−]	zeocin	HAT	6TG
−	sensitive	sensitive	resistant
+ ubiquitin-Ggpt2	resistant	resistant	sensitive
+ ubiquitin-Rgpt2	resistant	sensitive	resistant

Figure 1 Schematic representation of the mammalian split-ubiquitin assay

(a) An HT1080HPRT⁻ cell line expressing p65C-Cub-Rgpt2 is HAT-resistant (HAT^R) and 6TG-sensitive (6TG^S). (b and c) If Nub-hTBP1 is co-expressed, hTBP1 and p65C bind each other inside the living cell. As a result of this, ubiquitin is reconstituted and the p65C-Cub-Rgpt2 fusion protein is cleaved behind Cub. (d) The cleavage product Rgpt2 is subject to rapid degradation by the enzymes of the N-end rule, leading to HAT-sensitivity and 6TG-resistance.

carrying ubiquitin-Ggpt2 should have been HAT-resistant and 6TG-sensitive. As shown in Table 1 this prediction was confirmed. This demonstrated that the fusion protein was cleaved and that the cleavage product with the glycine was stable whereas the cleavage product with the arginine was degraded, following the N-end rule.

Next, we split ubiquitin in half and fused Nub to hTBP1, and Cub to the activation domain of human NF- κ B, p65C, and Rgpt2. A cell line expressing p65C-Cub-Rgpt2 alone should be HAT-resistant and 6TG-sensitive (Figure 1a). If hTBP1 and p65C interact *in vivo*, co-expression of Nub-hTBP1 should lead to a reversal of the phenotype, for hTBP1 should bind the activation domain of NF- κ B, which brings Nub and Cub in close contact (Figure 1b). The reconstituted ubiquitin will be recognized by the UBPs and Rgpt2 will subsequently be released from Cub (Figure 1c). As a consequence, Rgpt2 will be rapidly degraded, and the cell will become HAT-sensitive and 6TG-resistant (Figure 1d). A stable cell line expressing the p65C-Cub-Rgpt2 fusion was made by selecting the cells in zeocin, whose resistance marker was also present on the plasmid encoding the p65C fusion protein. This cell line was HAT-resistant and 6TG-sensitive, demonstrating that the fusion protein was enzymically active. Three independent stable cell lines were made from this one, co-expressing Nub alone

by selecting for the neomycin marker present on the Nub-expression plasmid. In all cases, the cell lines were still HAT-resistant and 6TG-sensitive (Figure 2, first column). However, when three independent stable cell lines were made co-expressing an Nub-hTBP1 fusion with p65C-Cub-Rgpt2, all cell lines became HAT-sensitive and 6TG-resistant (Figure 2, second column), demonstrating that hTBP1 interacts with p65C inside the living human cell.

We also utilized this system to verify the interaction between hSP100B and hHP1 α inside the living cell [17,20]. In this instance, hSP100B was fused to Nub, while hSP100B was fused to Cub-Rgpt2. Consistent with the *in vitro* interaction previously observed between hSP100B and hHP1 α [17], co-expression of these fusion proteins in our HT1080HPRT⁻ cell line resulted in a HAT-sensitive and 6TG-resistant phenotype (Figure 3). In conclusion, this result shows that these proteins associate with each other *in vivo*.

A Western-blot was performed with cell lines, expressing p65C-Cub-Rgpt2 together with Nub or with Nub-hTBP1, grown in nonselective medium (Figure 4). Epitopes for the HA tag were present in all of the fusion proteins. The p65C-Cub-Rgpt2 band was readily detected when Nub was present (lane 2). On the other hand, of the two cell lines co-expressing Nub-hTBP1 tested, both showed a reduction of this band, indicative of Rgpt2 protein degradation (lanes 3 and 4). For cell line number 2, which showed good expression of Nub-hTBP1, the p65C-Cub-Rgpt2 band was no longer detectable. For cell line number 1, where the Nub-hTBP1 expression was barely visible, the p65C-Cub-Rgpt2 band was reduced but still detectable. Therefore the amount of Nub-hTBP1 protein produced by the cell lines correlated with the degree of reduction of the p65C-Cub-Rgpt2 band. In conclusion, our results show that the Western-blot represents an independent method by which one can confirm protein interactions *in vivo*.

DISCUSSION

In the present study, we have successfully adapted the split-ubiquitin system for mammalian cells. We have used this assay to demonstrate that certain protein-protein interactions (between hTBP1 and NF- κ B and between hSP100B and hHP1 α) previously characterized *in vitro* also occur inside the living cell. We utilized a Cub-Rgpt2 fusion protein and a human fibroblast cell line which is HPRT-deficient. A stable cell line expressing a fusion of p65C [21] or hSP100B to Cub, followed by Rgpt2, is HAT-resistant and 6TG-sensitive. Co-expression of a fusion of hTBP1 or hHP1 α , respectively, to Nub results in a cell line that is HAT-sensitive and 6TG-resistant. This demonstrates that the system can be used to select for and to counterselect against protein interactions inside living human cells. The ubiquitin-processing machinery seems to be equally distributed in the cytosol of the cell [7], including the nucleus [8]; as a consequence, the assay is not limited to interactions that can be reconstituted in the nucleus of the cell. Also, the readout is based on proteolytic stability; hence transcription factors can be analysed [22]. We envisage this system being used in the following applications. (1) Two human proteins known to interact *in vitro* can be tested for interaction inside living human cells, as shown in the present study. (2) Libraries expressing altered forms of the proteins can be screened for mutants deficient in interaction. We propose to mutate the protein fused to Cub-Rgpt2, for the subsequent selection would demand a functionally active gpt2 protein; as a consequence, mutations leading to stop codons or frame shifts would not be selected for. (3) Cells expressing the two proteins of interest can be mutated and mutant cell lines, in which the two proteins no longer interact, can be selected for in HAT-medium.

Figure 2 hTBP1 interacts with p65C *in vivo*

Left column: An HT1080HPRT⁻ cell line co-expressing p65C-C_{ub}-Rgpt2 and N_{ub} was grown in DMEM/10% FCS (first line), in DMEM/10% FCS/HAT (second line), or in DMEM/10% FCS/6TG (third line). Right column: HT1080HPRT⁻ cell line co-expressing p65C-C_{ub}-Rgpt2 and N_{ub}-hTBP1 grown in different selection media as described above.

Since the cell lines are diploid the mutations are likely to be dominant and the genes can be cloned from cDNA expression libraries made from these mutant cell lines. If the two interacting proteins are part of a complex, for example it should be possible to clone the other components of the complex by this method. Also, the mutant cell lines can be used to analyse the function of the proteins of interest. (4) It is possible to perform competition screens in mammalian cells. In this scenario a cell line expressing the two proteins of interest as N_{ub} and C_{ub} fusions is transformed with a library expressing human cDNAs from a strong promoter. The cell line is originally HAT-sensitive because of the interaction of the two proteins. If a third protein competes with this

interaction by binding to one or both proteins, the clone expressing this protein can be isolated by its ability to confer HAT-resistance to the cell line. (5) It is possible to test the effects of drugs on specific protein interactions in human cells. (6) A library expressing fusions of N_{ub} to human cDNAs can be screened to find new partners for human proteins of interest.

Transcriptional regulation of human genes depends on a network of protein-protein interactions which occur between DNA-binding proteins and components of the transcriptional machinery. In the case of transcriptional activators, they regulate eukaryotic genes by simultaneously binding to enhancer sequences and targeting factors of the RNA polymerase II

Figure 3 hSP100B interacts with hHP1 α *in vivo*

Left column: The HT1080HPRT⁻ cell line co-expressing hSP100B-C_{ub}-Rgpt2 and N_{ub} was grown in DMEM/10% FCS (first line), in DMEM/10% FCS/HAT (second line) or in DMEM/10% FCS/6TG (third line). Right column: HT1080HPRT⁻ line co-expressing hSP100B-C_{ub}-Rgpt2 and N_{ub}-hHP1 α grown in different selection media as described above.

transcription complex [23]. Direct protein interaction between the activating region of the activator and the TBP has been proposed as an important step in transcriptional activation [24]. This proposition was confirmed by studies of activator mutants which showed a correlation between the strength of protein interactions *in vitro* and the activation capacity *in vivo* [25]. Also, artificial recruitment by fusing DNA-binding domains to components of the holoenzyme [26] results in transcriptional activation [27,28]. Recently two studies demonstrated that binding of TBP to the TATA-box is greatly enhanced upon activator binding in *S. cerevisiae* [14,15]. For human cells, we propose that NF- κ B binds the NF- κ B binding sites present in many promoters,

interacts with and thus recruits the TBP to the TATA-box, where it is needed to start transcription.

Likewise, protein-protein interaction has also been suggested to play an essential role in transcriptional repression [29]. An important class of repressors have also been shown to bind to specific promoter elements and concurrently interact with various targets (e.g. basal transcription factors, activator/coactivator proteins and co-repressors) to inhibit transcription. With regards to the hSP100B-hHP1 α interaction, previous studies have suggested that this interaction may be relevant in the ability of hHP1 α to repress transcription when tethered to DNA in human cells [17]. Other nuclear bodies, such as promyelocytic leukaemia

Figure 4 Western-blot confirming the *in vivo* interaction between p65C and hTBP1

Stable cell lines expressing the indicated fusions were grown in nonselective medium to show that the expression of the bands were observed in all control and experimental cell lines under identical conditions. The appearance of the Nub-hTBP1 band correlates with the disappearance of the p65C-Cub-Rgpt2 band. The protein band directly below that of Nub-hTBP1 is a non-specific band cross-reacting with the anti-HA antibody. The Western-blot for the hSP100B-hHP1 α interaction is not shown, because hSP100B-Cub-Rgpt2 was barely detectable with the anti-HA antibody. #1, cell line number 1; #2, cell line number 2.

protein ('PML') and hSP100A, a splice variant of hSP100B, have been demonstrated to be upregulated in the presence of interferons, which are important growth and tumour-suppressive cytokines [30,31]. It is tempting to speculate that the interaction between hSP100B and hHP1 α may be important in maintaining the physical and functional integrity of a transcriptional repressor complex. Further research is needed to examine which genes may be negatively regulated by this complex.

We thank Nils Johnsson for providing reagents and information prior to publication, as well as helpful discussions and suggestions, Mark Ptashne and Benno Müller-Hill for critical reading of the manuscript, Sandra Pellegrini for providing the HT1080HPRT⁻ cell line, Julia Fleck for technical help and Matthias Godde for help with the pictures of the human cells. This work was supported by grants from the Bundesministerium für Forschung und Bildung and the Max-Planck-Gesellschaft to N.L.

REFERENCES

- Ma, J. and Ptashne, M. (1988) Converting a eukaryotic transcriptional inhibitor into an activator. *Cell* **55**, 443–446.
- Fields, S. and Song, O. (1989) A novel genetic system to detect protein-protein interactions. *Nature (London)* **340**, 245–246.
- Luo, Y., Batalao, A., Zhou, H. and Zhu, L. (1997) Mammalian two-hybrid system: a complementary approach to the yeast two-hybrid system. *BioTechniques* **22**, 350–352.
- Aronheim, A., Zandi, E., Hennemann, H., Elledge, S. J. and Karin, M. (1997) Isolation of an AP-1 repressor by a novel method for detecting protein-protein interactions. *Mol. Cell. Biol.* **17**, 3094–3102.
- Maroun, M. and Aronheim, A. (1999) A novel *in vivo* assay for the analysis of protein-protein interaction. *Nucleic Acids Res.* **27**, e4.
- Johnsson, N. and Varshavsky, A. (1994) Split ubiquitin as a sensor of protein interactions *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 10340–10344.
- Varshavsky, A. (1997) The ubiquitin system. *Trends Biochem. Sci.* **22**, 383–387.
- Byrd, C., Turner, G. C. and Varshavsky, A. (1998) The N-end rule pathway controls the import of peptides through degradation of a transcriptional repressor. *EMBO J.* **17**, 269–277.
- Dünnwald, M., Varshavsky, A. and Johnsson, N. (1999) Detection of transient *in vivo* interactions between substrate and transporter during protein translocation into the endoplasmic reticulum. *Mol. Biol. Cell* **10**, 329–344.
- Witte, S., Lewke, N., Müller, S. and Johnsson, N. (1999) Probing the molecular environment of membrane proteins *in vivo*. *Mol. Biol. Cell* **10**, 2519–2530.
- Varshavsky, A. (1996) The N-end rule: functions, mysteries, uses. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 12142–12149.
- Kerr, L. D., Ransone, L. J., Wamsley, P., Schmitt, M. J., Boyer, T. G., Zhou, Q., Berk, A. J. and Verma, I. M. (1993) Association between proto-oncoprotein Rel and TATA-binding protein mediates transcriptional activation by NF-kappa B. *Nature (London)* **365**, 412–419.
- Schmitz, M. L., Stelzer, G., Altmann, H., Meisterernst, M. and Baeuerle, P. A. (1995) Interaction of the COOH-terminal transactivation domain of p65 NF-kappa B with TATA-binding protein, transcription factor IIB, and coactivators. *J. Biol. Chem.* **270**, 7219–7226.
- Li, X. Y., Virbasius, A., Zhu, X. and Green, M. R. (1999) Enhancement of TBP binding by activators and general transcription factors. *Nature (London)* **399**, 605–609.
- Kuras, L. and Struhl, K. (1999) Binding of TBP to promoters *in vivo* is stimulated by activators and requires Pol II holoenzyme. *Nature (London)* **399**, 609–613.
- Ptashne, M. and Gann, A. (1997) Transcriptional activation by recruitment. *Nature (London)* **386**, 569–577.
- Lehming, N., Le Saux, A., Schüller, J. and Ptashne, M. (1998) Chromatin components as part of a putative transcriptional repressing complex. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 7322–6.
- Ausubel, F., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1987) *Current protocols in molecular biology*, John Wiley & Sons, Inc., New York.
- Pellegrini, S., John, J., Shearer, M., Kerr, I. M. and Stark, G. R. (1989) Use of a selectable marker regulated by alpha interferon to obtain mutations in the signaling pathway. *Mol. Cell. Biol.* **9**, 4605–4612.
- Seeler, J. S., Marchio, A., Sitterlin, D., Transy, C. and Dejean, A. (1998) Interaction of SP100 with HP1 proteins: a link between the promyelocytic leukemia-associated nuclear bodies and the chromatin compartment. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 7316–7321.
- Moore, P. A., Ruben, S. M. and Rosen, C. A. (1993) Conservation of transcriptional activation functions of the NF-kappa B p50 and p65 subunits in mammalian cells and *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13**, 1666–1674.
- Wellhausen, A. and Lehming, N. (1999) Analysis of the *in vivo* interaction between a basic repressor and an acidic activator. *FEBS Lett.* **453**, 299–304.
- Ptashne, M. (1988) How eukaryotic transcriptional activators work. *Nature (London)* **335**, 683–689.
- Stargell, L. A. and Struhl, K. (1996) Mechanisms of transcriptional activation *in vivo*: two steps forward. *Trends Genet.* **12**, 311–315.
- Wu, Y., Reece, R. J. and Ptashne, M. (1996) Quantitation of putative activator-target affinities predicts transcriptional activating potentials. *EMBO J.* **15**, 3951–3963.
- Koleske, A. J. and Young, R. A. (1995) The RNA polymerase II holoenzyme and its implications for gene regulation. *Trends Biochem. Sci.* **20**, 113–116.
- Barberis, A., Pearlberg, J., Simkovich, N., Farrell, S., Reinagel, P., Bamdad, C., Sigal, G. and Ptashne, M. (1995) Contact with a component of the polymerase II holoenzyme suffices for gene activation. *Cell* **81**, 359–368.
- Gaudreau, L., Keaveney, M., Nevado, J., Zaman, Z., Bryant, G. O., Struhl, K. and Ptashne, M. (1999) Transcriptional activation by artificial recruitment in yeast is influenced by promoter architecture and downstream sequences. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 2668–2673.
- Hanna-Rose, W. and Hansen, U. (1996) Active repression mechanisms of eukaryotic transcription repressors. *Trends Genet.* **12**, 229–234.
- Lavau, C., Marchio, A., Fagioli, M., Jansen, J., Falini, B., Lebon, P., Grosveld, F., Pandolfi, P. P., Pelicci, P. G. and Dejean, A. (1995) The acute promyelocytic leukaemia-associated PML gene is induced by interferon. *Oncogene* **11**, 871–876.
- Guldner, H. H., Szosteki, C., Grotzinger, T. and Will, H. (1992) IFN enhance expression of Sp100, an autoantigen in primary biliary cirrhosis. *J. Immunol.* **149**, 4067–4073.