
Regulatory elements involved in the bidirectional activity of an immunoglobulin promoter

Noëlle Doyen, Marc Dreyfus and François Rougeon

Unité de Génétique et Biochimie du Développement, LA CNRS 361, Département d'Immunologie, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France

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ABSTRACT

We show that the promoter from the mouse VH441 heavy-chain immunoglobulin gene, when present on plasmids transiently introduced into myeloma cells, promotes transcription bidirectionally, due to the presence on both strands of TATA-like sequences bracketting the highly conserved decanucleotide element. The two divergent promoters compete for the transcriptional machinery, their relative strength ultimately reflecting the likeness of the two TATA boxes to the consensus sequence. Moreover, their relative activity is also strongly influenced by certain point mutations within the distally located heavy-chain enhancer. The bearing of these results on current concepts of promoter function is discussed.

INTRODUCTION

Two major classes of cis-acting elements are required for the efficient initiation of transcription by RNA polymerase II, i.e. promoters and enhancers (reviewed in 1-3). Promoters are short DNA regions (< 200 bp) which are located immediately upstream of transcription units and contain all the information required for an accurate initiation *in vivo*, whereas enhancers potentiate the activity of nearby promoters, mainly irrespective of both orientation and distance. Promoters can usually be further dissected into the "TATA" box (consensus sequence TATAAA ; cf 4) and the "upstream promoter element" (UPE), which are located 25-30 and 40-110 bp upstream from the cap site, respectively. The TATA element presumably serves to identify the sense strand, as well as the precise transcription start point (5). In contrast, the UPEs, which in many cases endows the promoter with its inductibility and/or tissue-specificity, can frequently be varied in position and/or orientation without much effect on promoter strength.

Perhaps not unexpectedly given these properties of UPEs, many promoters have been found to cause transcription from both DNA stands. These bidirectional promoters either are bracketted by divergently orientated TATA-like elements (6-8) or consist in UPEs which promote transcription even in the absence of an associated TATA box (9-12). Several studies have analyzed the factors governing the relative strength of the two divergent promoters. It appears that, at least in some cases, competition takes place between them, since the deletion of the TATA box or cap site from one strand stimulates

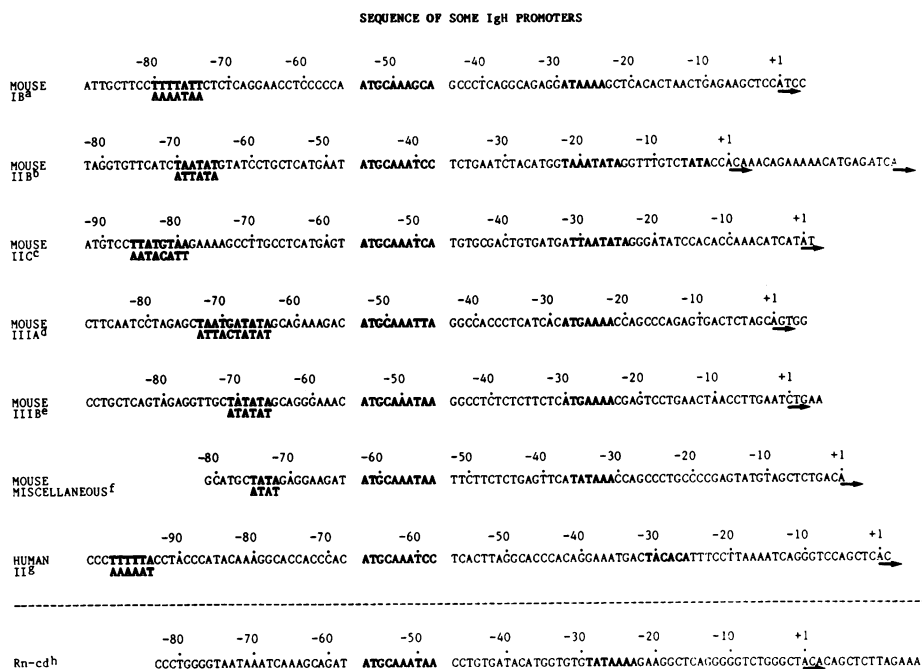


Figure 1 : Sequence of some heavy-chain promoters for which the precise transcription start point has been mapped. Only one gene is listed for each of the different V_H subgroups, which are named according to Ref. 41. The cd element, as well as possible TATA boxes on both DNA strands, are indicated in boldface. The sequences are numbered from the main transcription start point, which is indicated in each case by a closed arrow. Positive numbers correspond to the regions transcribed in the heavy-chain mRNA. The VH441 promoter studied here is shown under the heading IIIB. The sequence of the renin-cd chimaeric promoter (Rn-cd) is also shown for comparison.^a Ref. 42 ; ^b Ref. 43 ; ^c Ref. 15 ; ^d Ref. 44 ; ^e Ref. 17 and this work; ^f Ref. 45 ; ^g Ref. 46 ; ^h Ref. 22

opposite transcription (8, 13). The generality of this feature remains to be established. Moreover, we ignore whether other, more distally located *cis*-acting elements such as enhancers, can affect the balance between the divergent promoters : different promoters have been shown to respond preferentially to certain enhancers compared to others (14), but it is not known whether this also applies to overlapping promoters that share regulatory elements.

The promoters of the immunoglobulin heavy chain genes (IgH) only direct transcription in cells of the B lymphocyte lineage, even if associated with an enhancer which is active in many cell types (15-17). These promoters contain an UPE which fits closely to the consensus sequence ATGCAAATNA, and is here referred to as "cd" as in Ref. (18). The cd element is present in reverse orientation (dc) in the promoters of

immunoglobulin κ light chain genes, which are also B-specific ; moreover, a dc element is also part of the heavy-chain enhancer (18, 19). Together with an associated TATA box, the cd/dc element is both necessary and sufficient for the activity of immunoglobulin promoters in B cells (20-23) and it has recently been shown to be the target for a B-cell specific activator (24, 25).

During the course of our work (17, 22) we noted that, like many other heavy-chain promoters, the subgroup IIIB mouse promoter which we used as a reference, carried a divergently orientated TATA-like box upstream of the cd element, in addition to the "physiological" TATA box (Fig. 1). Given the orientation-independent character of the cd element, we conjectured that it might promote transcription bidirectionally. Here we show that such is the case and we also examine some of the elements which affect the balance between the two directions of transcription.

MATERIALS AND METHODS

Techniques for plasmid construction, oligonucleotide directed mutagenesis, culture and transfection of the myeloma cell line X63Ag8, as well as chloramphenicol acetyltransferase (CAT) assays, have been described (17, 22). Similarly, the transcription start point of the cat gene was mapped using primer extension as described previously (22)

The origin of the DNA fragments used in this work has been already described. Briefly, the 1 kb Xba1-Xba1 fragment encompassing the heavy chain (IG) enhancer, and the 220 bp fragment carrying the promoter of the VH441 gene (V1 fragment) were described (17). The renin-cd promoter (Rn-cd) used here is the R6-cd fragment (22) : it extends from -83 to +30 with respect to the renin transcription start point. The tk promoter (nt.-196 to +52 with respect to the start) has been described in (17). For inversion experiments, promoter fragments were provided at both ends with Hind III linkers and cloned in either orientations in the Hind III site of pSBI-IG, a derivative of pSBI (27) carrying the IG enhancer in the BamH1 site. In the various constructs, the cat initiation codon was the first AUG encountered downstream from the cd (or dc) element, except in the reversed tk fragment which carries an AUG codon in phase with the cat gene. However, adding extra aminoacids to the N terminal of chloramphenicol acetyltransferase does not usually impairs its activity (P. Herbomel, personal communication).

RESULTS

Bidirectionality of the VH441 promoter

In our previous work, we showed that a DNA fragment extending from nucleotides -118 to +59 with respect to the transcription start site of the VH441 gene (mouse subgroup IIIB), behaves as an efficient promoter in myeloma cells (17, 22). This fragment,

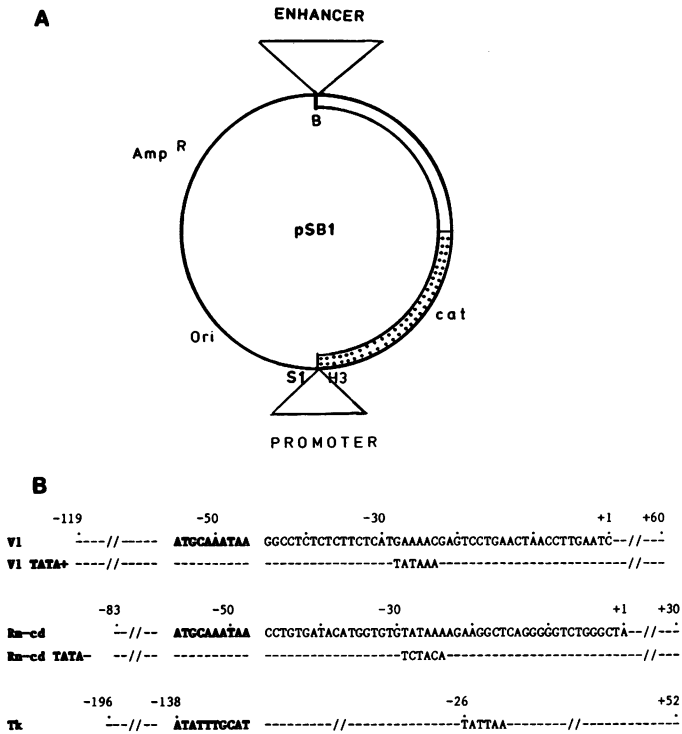


Figure 2 : (A) : General structure of the plasmids used in this work. The starting plasmid, pSB1 (27) is similar to pSV0 CAT (28), except that it lacks the pBR322 "poison" sequences. Various promoters and enhancers are inserted upstream and downstream from the *cat* gene, respectively. Thin line : pBR322 sequences. Stippled box : *cat* gene. Open box: SV40 sequences. H3, S1 and B stand for *Hind*III, *Sal*I and *Bam*H1 cleavage sites, respectively. (B) : Schematic structure of three promoter fragments used in this work, the origin of which is described in Materials and Methods. The mutated fragment V1-TATA⁺ and Rn-cd-TATA⁻ are shown schematically below their unmutated counterparts. Numbering is from the main transcription start point, as determined in (22) for Rn-cd, in (26) for Tk, and in this work for V1. The cd/dc element is indicated in boldface.

hereafter referred to as V1, bears a perfectly canonical cd, but its TATA homology is not particularly striking, an A-rich element TGAAAA being found in the same location as the TATA box of most heavy-chain promoters (Fig. 1). V1 also carries the TATA-like sequence TATATA, 11 nucleotides upstream from the cd element, on the opposite strand (Fig. 1).

To assess whether this promoter could work in both directions, the fragment was inserted in either orientation into pSB1-IG, a promoterless *cat* plasmid bearing the immunoglobulin heavy chain enhancer (Fig. 2A). The corresponding constructs were transfected into the mouse myeloma cells X63Ag8, and the transient CAT activity which

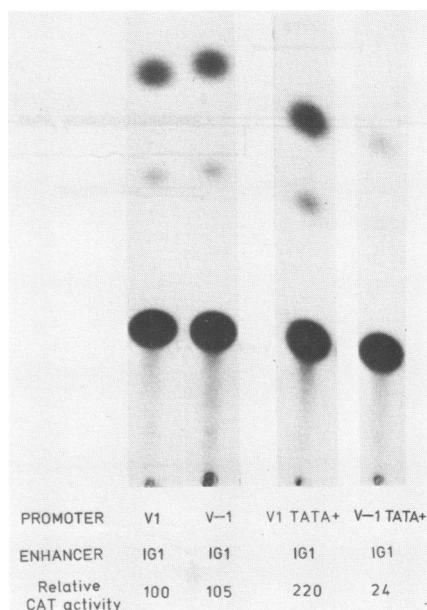


Figure 3 : CAT activity in extracts of myeloma cells (X63Ag8) harvested 48 h after transfection with various plasmids. The promoter (first line) and the enhancer (second line) present in each construction are indicated under the corresponding lane. IG1 stands for the immunoglobulin heavy-chain enhancer (1Kb *Xba*1-*Xba*1 fragment : see Materials and Methods) in the same orientation with respect to transcription as in the heavy-chain gene. The lower line indicate relative CAT activities (V1 promoter and IG1 enhancer = 100), averaged from two to five independent experiments.

developed over a period of 48 h was taken as a measure of promoter strength (28). As seen in Fig. 3, a nearly equal CAT activity was recorded for the "sense" or "antisense" orientations of the fragment (denoted V1 and V-1, respectively), thus demonstrating the bidirectionality of this promoter. Using primer extension (22), the main transcription start points were mapped at 46 and 40 nt downstream from the cd/dc element for the "sense" and "antisense" transcription, respectively, i.e. at the expected distance from the TATA-like element in each case (Fig. 4). The fact that bidirectionality rests on the presence of these TATA elements, was further demonstrated by inverting two promoters which carry a cd/dc element, but lack an antisense TATA box. We have previously inserted the cd element within the promoter of the mouse renin 1 gene (Rn-cd promoter, see Ref. 22). Moreover, a dc element occurs naturally within the Herpes virus thymidine kinase (*tk*) promoter (26). These promoters were inserted in either orientations into pSB1-IG but in neither case did the "antisense" transcription exceed 1 % of the "sense" transcription (not shown)

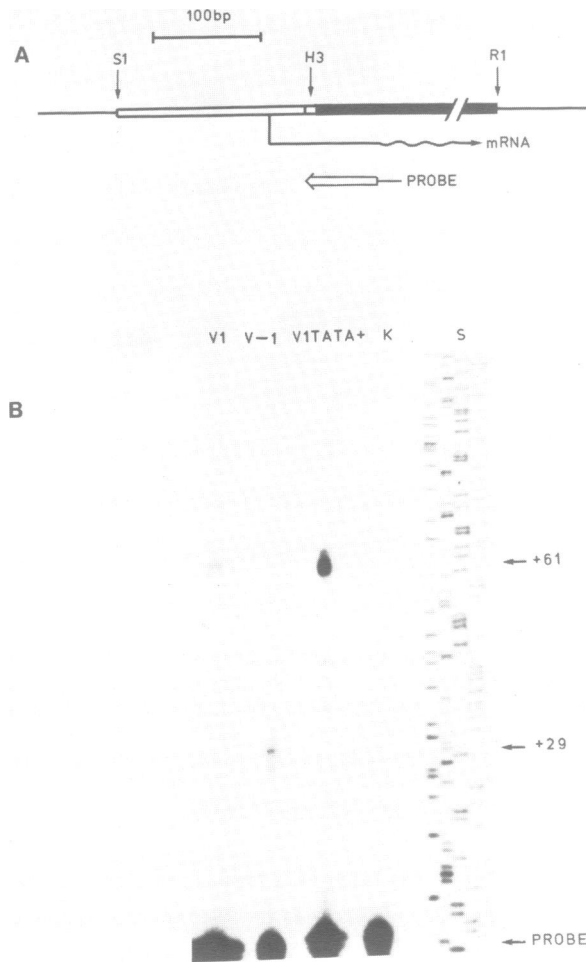


Figure 4 : Mapping of the transcription start point from the two divergent VH441 promoters. (A) : Strategy for the preparation of the single-stranded DNA probe. A 450 bp *Sal*I-*Eco*R1 fragment corresponding to the V1 fragment followed by the first third of the *cat* gene (Fig. 2) was subcloned into M13 mp 9 and used to prepare a single stranded, uniformly labelled DNA probe (open arrow) complementary to position 1-71 with respect to the most 5' nucleotide of the *Hind* III site. Thin line : M13 DNA. Open box : V1 fragment followed by the *Hind* III linker. Black box : *cat* sequences. Wavy line : mRNA. R1 stands for *Eco*R1, other symbols as in Fig. 1. (B) : Primer extension mapping. Myeloma cells were transfected with pSB1-IG plasmids carrying the indicated promoter. Poly(A)⁺ mRNA (20 µg), extracted 40 h later, was hybridized with an excess of single-stranded probe and the hybrids were elongated with reverse transcriptase in the presence of all four dNTP and analyzed on a sequencing gel. The figures +29 and +61 indicate the distance between the main transcription start observed here, and the 3' end of the probe. In lane K, the probe was hybridized with 20 µg of mouse kidney poly(A)⁺ mRNA. S : calibrating sequencing lanes.

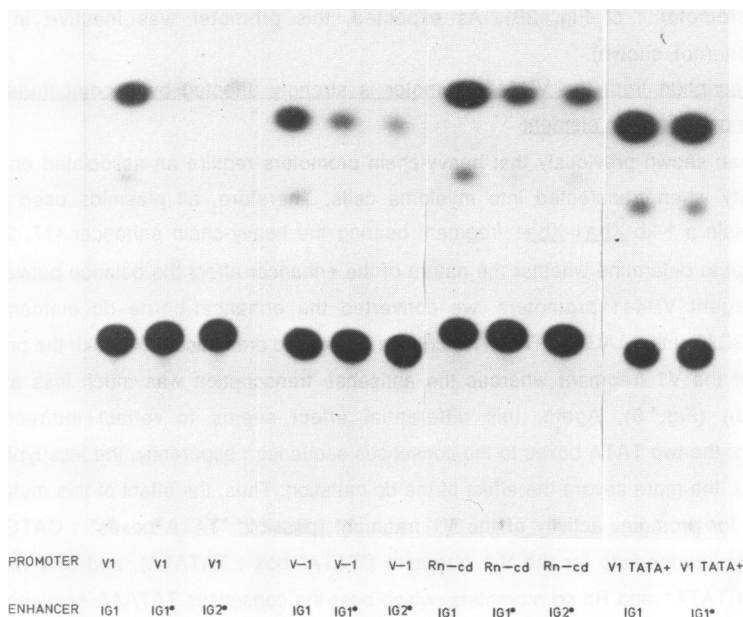


Figure 5 : Effect of the dc enhancer mutation on the activity of various promoters. IG1* and IG2* refer to the two possible orientations of the mutated enhancer with respect to the direction of transcription, with "1" referring to the orientation found in the heavy-chain gene. Other notations as in Fig. 3.

Competition between the two directions of transcription from the VH441 promoter

To establish whether the two divergent promoters direct transcription independently or compete with one another, the canonical TATAAA element was substituted for the natural sequence ranging from 18 to 23 nt downstream from the cd element, i.e. at a position typical for the TATA box in other heavy-chain promoters (this engineered fragment is referred to as V1 TATA⁺ in Fig. 2B). The substitution increased promoter strength two-fold (Fig. 3) and did not alter the main transcription start point (Fig. 4). However, when inverted, this promoter (V-1 TATA⁺ in Fig. 3) proved much weaker than V-1. As a result, the total transcription from both strands remains approximately constant for the V1 and V1 TATA⁺ fragments. Apparently, the divergent promoters compete for the transcriptional machinery, the balance between them reflecting the closeness of the two TATA boxes to the consensus sequence.

As stated above, the Rn-cd or tk promoters failed to promote transcription in the "antisense" direction. To insure that these inverted promoters are truly inactive rather than simply silenced by an unfavorable competition with the "sense" transcription, we constructed a derivative of the Rn-cd promoter carrying an altered TATA box (Rn-cd

TATA- promoter : cf Fig. 2B). As expected, this promoter was inactive in either orientation (not shown).

The transcription from the VH441 promoter is strongly affected by a point mutation in the enhancer-borne dc element

It has been shown previously that heavy-chain promoters require an associated enhancer for activity when transfected into myeloma cells. Therefore, all plasmids used in this work contain a 1 kb Xba1-Xba1 fragment bearing the heavy-chain enhancer (17, 22). As an attempt to determine whether the nature of the enhancer affect the balance between the two divergent VH441 promoters, we converted the enhancer-borne dc element from TAATTTGCAT into TAAGATCTAT. This change depressed dramatically (40 fold) the promoter activity of the V1 fragment whereas the antisense transcription was much less affected (4-5 fold), (Fig. 5). Again, this differential effect seems to reflect indirectly the likeness of the two TATA boxes to the consensus sequence : apparently, the less typical the TATA box, the more severe the effect of the dc mutation. Thus, the effect of this mutation is dramatic for promoter activity of the V1 fragment (possible "TATA boxes" : CATGAA or TGAAAA), intermediate for the V-1 fragment ("TATA" box : TATATA), and only marginal for the V1 TATA⁺ and Rn-cd promoters, which bear the consensus TATAAA sequence (Fig. 5).

DISCUSSION

In this work, we have shown that the promoter of the VH441 mouse heavy-chain gene, acts-bidirectionally due to the presence on both DNA strands of TATA-like boxes bracketting the cd/dc element. Modification of one of the TATA boxes affects the strength of both promoters in opposite ways, demonstrating that they do not work independently, but in competition. Finally, we report that a point mutation within the dc element borne by the associated heavy-chain enhancer alters 10 fold the balance between the two directions of transcription. The bearings of these different points will be separately discussed.

Competition between the two divergent VH441 promoters

The replacement of the upper strand TATA-like element TGAAAA by the canonical TATAAA not only results in a two-fold increase in the sense transcription but also impairs the antisense transcription. In this respect, the VH441 promoter resembles other systems in which overlapping promoters share common UPE(s) : thus, in the two SV40 early promoters EEP and LEP, the disruption of the EEP TATA box stimulates transcription from LEP (29). In the chicken skeletal α -actin promoter, which exhibits bidirectionality, antisense transcription greatly increases after deletion of the sense TATA box (8), and the same holds for the adonovirus IV a2 gene, which cannot be transcribed in vitro unless the cap site from the divergent major late promoter is deleted (13). We hypothesize that, in these systems, the overlapping promoters compete for the transcriptional machinery :

conceivably, in the case of the VH441 promoter, the cd-bound factor might have a single binding site for some other element of the transcriptional machinery (perhaps the TATA binding factor : see below), so that transcription cannot be initiated on both strands simultaneously.

Whatever the interpretation, these data show that the effect of deletions or mutations on promoter activity should be interpreted with caution : would the alteration of a particular sequence increase promoter strength, this sequence could be part of an overlooked competing promoter, rather than the target for a repressor, as usually assumed.

The activity of the VH441 promoter is dependent upon the presence of an intact dc element in the heavy-chain enhancer

Altering the enhancer-borne dc element impairs the sense transcription, while affecting only moderately the antisense one. Again, this differential effect presumably reflects the nature of the two divergent TATA boxes : the activity of cd/dc dependent promoters bearing "consensus" TATA boxes seem generally less sensitive to the dc mutation than that of "non consensus" promoters (Fig. 5).

The striking effect of the dc point mutation on the activity of the VH441 promoters was unexpected : natural enhancers carry multiple binding sites for transcriptional activators, the function of which is largely redundant (30, 31), and indeed, previous mutational analyses (32-34) of the heavy-chain enhancer have failed to reveal any crucial role of the dc element, provided that sufficiently long versions of the enhancer are used, as here. However, in these studies, the dc-deficient enhancer was assayed in conjunction with conventional promoters unrelated to the immunoglobulin genes, rather than with cd/dc dependent promoters.

The presence of the dc sequence, i.e. of a promoter element, within the heavy-chain enhancer has long been an intriguing and, until recently, unique feature of the heavy-chain gene (but see 35). Following procaryotic precedents, Ptashne suggested that the fixation of the dc-binding factor to the enhancer might in some way facilitate its productive binding to the promoter (36, 37). Our results (Fig. 5) suggest that the enhancer-borne dc element is not actually necessary for heavy-chain promoter function, unless the TATA box diverges from the consensus. Although a definitive molecular interpretation of these observations will ultimately require mechanistic studies with purified components, we suggest that the transcriptional factor(s) bound to the cd/dc element can make specific contacts with the TATA-binding protein, and that two of these contacts are required to compensate for a weak interaction between the TATA-binding factor and its non-consensus target. Indeed, such direct interactions between factors bound to the TATA box and to UPEs have been documented in several cases (38-40).

Whatever its exact mechanism, the differential effects of the dc mutation on the

activity of the divergent VH441 promoters, or that of the derivative carrying the consensus TATAAA sequence, illustrate quite dramatically the emerging concept that each promoter will respond preferentially to certain enhancers compared to others (14). As documented here, this holds even for promoters carrying the same UPE, but differing in the region of TATA homology.

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REFERENCES

- 1 McKnight, S. and Tjian, R (1986) *Cell* **46**, 795-805
- 2 Matthias, P., Gerster, T., Bohmann, D. and Schaffner, W. in *Nucleic Acids and Molecular Biology Vol. 1*, F. Eckstein and D. Lilley Eds, Springer-Verlag, Berlin 1987
- 3 Guarente, L. (1988) *Cell* **52**, 303-305
- 4 Bücher, P. and Trifonov, E.N. (1986) *Nucl. Acids Res.* **14**, 10009-10026
- 5 Breahtnach, R. and Chambon, P. (1981) *Annu. Rev. Biochem.* **50**, 349-383
- 6 Struhl, K. (1986) *Mol. Cell. Biol.* **6**, 3847-3853
- 7 Efrat, S. and Hanahan, D. (1987) *Mol. Cell. Biol.* **7**, 192-198
- 8 Grichnik, J.M., French, B.A. and Schwartz, R.J. (1988) *Mol. Cell. Biol.* **8**, 4587-4597
- 9 Ogden, J., Stanway, C., Kim, S., Mellor, J., Kingsman, A.J. and Kingsman, S.M. (1986) *Mol. Cell. Biol.* **6**, 4335-4343
- 10 Crouse, G.F., Leys, E.J., McEwan, R.N., Frayne, E.G. and Kellems, R.E. (1985) *Mol. Cell. Biol.* **5**, 1847-1858
- 11 Williams, T.J. and Fried, M. (1986) *Mol. Cell. Biol.* **6**, 4558-4569
- 12 Dynan, W.S. (1986) *Trends in Genet.* **2**, 196-197
- 13 Natarajan, V., Madden, M.J and Salzman, N.P. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6290-6294
- 14 Garcia, J.V., Bich-Thuy, L.T., Stafford, J. and Queen, C. (1986) *Nature* **322**, 383-385
- 15 Grosschedl, R. and Baltimore, D. (1985) *Cell* **41**, 885-897
- 16 Mason, J.O, Williams, G.T. and Neuberger, M.S. (1985) *Cell* **41**, 479-487
- 17 Doyen, N. Leblond-Francillard, M., Holm, I., Dreyfus, M. and Rougeon, F. (1986) *Gene* **50**, 321-331
- 18 Falkner, F.G. and Zachau, H.G. (1984) *Nature* **310**, 71-74
- 19 Parslow, T.G., Blair, D.L., Murphy, W.J. and Granner, D.K. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2650-2654
- 20 Ballard, D. and Bothwell, A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9626-9630
- 21 Queen, C., Foster, J., Stauber, C. and Stafford, J. (1986) *Immunol. Rev.* **89**, 49-68
- 22 Dreyfus, M., Doyen, N. and Rougeon, F. (1987) *EMBO J.* **6**, 1685-1690

- 23 Wirth, T., Staudt, L. and Baltimore, D. (1987) *Nature* 329, 174-178
- 24 Müller, M.M., Ruppert, S., Schaffner, W. and Matthias, P. (1988) *Nature* 336, 544-551
- 25 Scheidereit, C., Cromlish, J.A., Gerster, T., Kawakami, K., Balmaceda, C.G., Currie, R.A. and Roeder, R.G. (1988) *Nature* 336, 551-557
- 26 Parslow, T.G., Jones, S.D., Bond, B. and Yamamoto, K.R. (1987) *Science* 235, 1498-1501
- 27 Herbomel, P., Bourachot, B. and Yaniv, M. (1984) *Cell* 39, 653-662.
- 28 Gorman, C.M., Moffat, L.F. and Howard, B.H. (1982) *Mol. Cell Biol.* 2, 1044-1051
- 29 Wasylyk, B., Wasylyk, C., Matthes, H., Wintzerich, M. and Chambon, P. (1983) *EMBO J.*2, 1605-1611.
- 30 Schaffner, G., Schrim, S. Müller-Baden, B., Weber, F. and Schaffner, W. (1988) *J. Mol. Biol.* 201, 81-90
- 31 Ondek, B., Gloss, L. and Herr, W. (1988) *Nature* 333, 40-45
- 32 Kadesch, T., Zervos, P. and Ruezinsky, D. (1986) *Nucl. Acids Res.* 14, 8209-8221
- 33 Lenardo, M., Pierce, J.W. and Baltimore, D. (1987) *Science* 236, 1573-1577
- 34 Kiledjian, M., Su, L.K. and Kadesch, T. (1988) *Mol. Cell. Biol.* 8, 145-152
- 35 Johnson, P.F., Landschulz, W.H., Graves, B.J. and McKnight, S.L. (1987) *Genes Dev.* 1, 133-146
- 36 Ptashne, M. (1986) *Nature* 322, 697-701
- 37 Ptashne, M. (1988) *Nature* 335, 683-689
- 38 Sawadogo, M. and Roeder, R.G. (1985) *Cell* 43, 165-175
- 39 Horikoshi, M., Carey, M.F., Kakidani, H. and Roeder, R.G. (1988) *Cell* 54, 665-669
- 40 Horikoshi, M., Hai, T., Lin, Y.S., Green, M.R. and Roeder, R.G. (1988) *Cell* 54, 1033-1042
- 41 Kabat, E.A., Wu, T.T., Bilofsky, H., Reid-Miller, M. and Perry, H. in "Sequences of Proteins of immunological interest" NIH, 1983
- 42 Kataoka, T., Nikaïdo, T., Miyata, T., Moriwaki, K. and Honjo, T. (1982) *J. Biol. Chem.* 257, 277-285
- 43 Neuberger, M.S. (1983) *EMBO J.* 2, 1373-1378
- 44 Clarke, C., Berenson, J., Gorman, J., Boyer, P.D., Crews, S, Siu, G. and Calame, K. (1982) *Nucl. Acids Res.* 10, 7731-7749
- 45 Kudo, A., Nishimura, Y. and Watanabe, T. (1985a) *J. Immunology* 135, 642-645.
- 46 Kudo, A., Ishihara, T., Nishimura, Y. and Watanabe, T. (1985b) *Gene* 33, 181-189