Identification of USF as the ubiquitous murine factor that binds to and stimulates transcription from the immunoglobulin λ 2-chain promoter

Ling A.Chang, Tammy Smith, Philippe Pognonec¹, Robert G.Roeder¹ and Helios Murialdo*

Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada and ¹Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021, USA

Received September 18, 1991; Revised and Accepted December 16, 1991

ABSTRACT

To study the specificity and identity of NF- λ 2, a ubiquitous murine nuclear factor that interacts specifically with the promoter of the λ 2-chain gene and stimulates its transcription, competition experiments were carried out using DNA fragments from various immunoglobulin regulatory elements. The results showed that a fragment containing the H-chain enhancer competed efficiently for the binding of NF- λ 2. Dissection of the H-chain enhancer revealed that the μ E3 motif contributed the competing ability. Additionally, a regulatory region found in the adenovirus major late promoter, which interacts with the human general transcription factor USF, competed very efficiently for binding to NF- λ 2. This region contains a sequence, CACGTGAC, which is identical to a region within the NF- λ 2 motif. The pattern of complexes formation using oligonucleotide probes corresponding to the NF- λ 2 and USF motifs were identical, and they both differed from that displayed by the E3 probes. Antisera against different domains of USF also react specifically with NF- λ 2 showing that this factor is antigenically related, if not identical, to USF. Furthermore, the activity of the λ 2 promoter in an *in vitro* transcription assay was significantly reduced when the nuclear extract used was USF-depleted. Addition of exogenous USF to this extract restored the transcription activity. Therefore, we conclude that NF- λ 2 is the murine homologue of USF.

INTRODUCTION

The specificity of immunoglobulin gene expression, which is restricted to cells of the B-lymphoid lineage, is controlled by the promoter and one or more enhancers. The two enhancers of the H- and κ -chain genes are located in their major introns (1, 2, 3, 4, 5) and downstream of C α and C κ , respectively (6, 7). Recently, the enhancers for the λ -chain genes, one for each cluster of constant gene segments, have been localized to 15.5 kb and

35 kb downstream of C λ 4 and C λ 1, respectively (8). As opposed to the H- and κ -chain genes, the λ -chain genes lack an enhancer in the major intron (3, 9).

The promoter and the enhancers contain multiple sequence elements (or motifs) to which nuclear factors bind. Among these are the octamer (*O*) and the so called *E* motifs (6, 7, 10, 11, 12, 13, 14). *O* is the binding site for the ubiquitous factor Oct-1 and the B-cell-specific factor Oct-2 (15, 16, 17). The *O* motif is present in the promoter of all immunoglobulin genes and in the intronic and downstream enhancers of the H-chain gene. A variant of it is also found in the κ -chain intronic enhancer (18, 19). The *E* motifs, of which there are several in the H- and κ -chain enhancers, comprise a series (μ Ei and κ Ei), all variations of the general consensus sequence CA(T/G)NTGG(T/C). The *E*-binding proteins are all ubiquitous (20, 21). So far, the only motifs identified in the regulatory elements of the λ -chain genes include *O* in the promoters and a sequence in the enhancers that resembles μ E2 (8).

We are interested in identifying the nuclear factors and the binding sites that contribute to the regulation of the expression of the murine λ -chain genes. We have previously reported a ubiquitous transcriptional nuclear factor, called NF- λ 2, which interacts with a 14-bp palindromic sequence located 30 bp upstream of *O* in the immunoglobulin λ 2-chain promoter, and in so doing, stimulates the basal level of expression of the λ 2-chain gene (22). Here we report the binding specificity and the identity of NF- λ 2.

MATERIALS AND METHODS

Cell lines, media and nuclear extract preparation

The mouse variant plasmacytoma cell line J558L was grown in Dulbecco modified Eagle medium containing 10% newborn calf serum, 40 μ M β -mercaptoethanol, and penicillin and streptomycin sulfate. Nuclear extracts were made according to published procedures (22).

Cell culture and nuclear extract of Namalwas cells followed procedure described previously (18).

* To whom correspondence should be addressed

Probe and competitor DNA fragments

The original source of DNA for the preparation of fragments $\lambda 2p$ and κe was described previously (22) (see Fig. 1Aa and 1Ae); $\kappa mopc41p$ competitor (κp) (see Fig. 1Ab) was obtained from λ gtWES-Vmopc41, which contains the unrearranged V gene segment counterpart of the one expressed in the MOPC41 myeloma (23); Hmopc315 competitor (Hp) (see Fig. 1Ac) was obtained from pMOPC315-VH, which contains the rearranged V gene segment of the α -chain gene of MOPC315 (24) (a gift of A. Rinfret, K. J. Dorrington and M. Klein, University of Toronto); He competitor (see Fig. 1Ad) was obtained from pUC19He which contains the *Xba*I-*Eco*RI fragment of the H-chain enhancer (2). This fragment was digested with *Pvu*II to generate a 381-bp fragment which contains the *E1* and part of the *E2* motifs, and a 302-bp fragment which contains the *E3*, the *E4* and the *O* motifs. A 51-bp *Dde*I-*Hinf*I fragment encompassing the *E4* and *O* motifs (see Fig. 1Ad) was obtained from pUC19-51bp (25) (a gift from H. Yu, L. Shen and L. A. Eckhardt, Columbia University). A NF- $\lambda 2$ motif (NF- $\lambda 2M$) probe (and competitor) was obtained by annealing oligonucleotides 5'-G-TAGATCTCACGTGACATCTTATCTGCA3' and 5'-GATAA-GATGTCACGTGAGATCTACTGCA3' (see Fig. 3A); a *USF* binding site (*USF-BS*) probe (and competitor) was obtained by annealing oligonucleotides 5'-GGTGTAGGCCACGTGACCGG-GTGCTGCA3' and 5'-GCACCCGGTCACGTGGCCTACACCTGCA3' (see Fig. 3A); a $\mu E3$ probe (and competitor) was obtained by annealing oligonucleotides 5'-TGCAGCAGGTCA-TGTGGCAAGGC3' and 5'-TGCAGCCTTGCCACATGACCTGCA3' (see Fig. 3A); a *E3* probe (and competitor) was obtained by annealing oligonucleotides 5'-TCGACTTGTCCTATGTGG-TTACAG3' and 5'-TCGACTGTAACCACATGGGACAAG3' (see Fig. 3A); a 76-bp *Hind*III-*Eco*RI *O*-containing fragment, used as competitor, was obtained from pUC19OCT (22).

Portions of the cloned fragments in the above vectors were subcloned and modified using standard recombinant DNA techniques (26). Appropriate fragments were excised by cleavage with restriction enzymes from various sources and purified by polyacrylamide gel electrophoresis.

End labelling, band retardation-competition analysis and interaction with anti-USF antibody

End labelling, band retardation and competition assays followed the published procedures (22). Briefly, the binding reaction contained 50 mM Tris-HCl (pH 7.9), 60 mM KCl, 5 mM DTT, 4% PEG-8000, 5% glycerol, 2 μ g poly(dI-dC).poly(dI-dC), 5,000 to 8,000 cpm of probe, and 5 to 10 μ g of nuclear extract in a total volume of 16 μ l. Competition reactions were carried out using the conditions described above with the addition of various amount of competitor DNA. The reaction was initiated by adding the nuclear extract to the mixture. To detect interaction between DNA-protein complexes and antisera, the anti-USF serum was added to the DNA-extract mixture upon completion of the binding reaction. The mixture was further incubated for 10 min at room temperature before electrophoresis in a polyacrylamide gel.

In vitro transcription and translation assay

The procedure for the *in vitro* transcription and translation of USF and TFE3 cDNAs (30) (a kind gift from H. Beckmann and T. Kadesch, University of Pennsylvania School of Medicine) followed the published procedure (31).

In vitro transcription assay

The *in vitro* transcription assay followed the published procedures (27). 250 ng of supercoiled plasmid p $\lambda 2$ (9), which contains the rearranged $\lambda 2$ -chain gene, was used as template with 7 μ g

Fig. 1. (A) Fragments of DNA used as probes and competitors. The original source of DNA fragments was described in Methods and Materials. They correspond to the promoter regions of a) the $\lambda 2$ -chain gene ($\lambda 2p$), b) the $\kappa mopc41$ -chain gene (κp), c) the α -mopc315-chain gene (Hp) and the intronic enhancer regions of d) the heavy chain (He) and e) the κ chain (κe). In diagrams a and b the first nucleotide of the transcript is +1 (4, 9). In c the position assigned for the startsite is the most likely one based on the published data (38, 39). L, V, D, and J correspond to sections coding for leader, variable, diversity and joining gene segments, respectively. Restriction sites in parentheses were introduced by insertion of linkers. The fragments used as probes and competitors are represented as thick lines below the restriction maps. (B) Competition for complex formation by various transcriptional regulatory elements. $\lambda 2p$ was used as a probe in competition experiments with a nuclear extract prepared from J558L. The molar ratios of probe to competitor are indicated above the lanes. The competitor DNAs used are indicated above the brackets. Absence of competitor DNA in the binding reaction is also shown (lane none).

of Namalwa or USF-depleted Namalwa nuclear extracts. To reconstitute the depleted extract, 50 ng of USF (43 kD) purified from bacterial extracts was added to the depleted reaction mixture. *In vitro*-synthesized RNAs were analyzed by primer extension reaction with reverse transcriptase. The probe used (GCTGACCTGAGCAGAGAGCC) for mapping the $\lambda 2$ transcript contains sequences from position +73 to +92 of the $\lambda 2$ -chain gene with respect to the transcription startsite (9).

Generation and preparation of anti-USF antibody, USF depletion of Namalwa nuclear extract, and purification of 43 kD USF from bacterial extracts

All procedures followed the methods published previously (27).

RESULTS AND DISCUSSION

Regulatory regions of other immunoglobulin genes contain a NF- $\lambda 2$ -like binding site

To investigate whether any sequence motif with potential regulatory function present in the promoters and enhancers of other immunoglobulin genes can act as ligand for NF- $\lambda 2$, a series of competition assays were carried out. Fig. 1A shows the genetic elements and pertinent restriction sites present in the DNA segments used as competitors in the binding assays. We have previously shown that incubation of the $\lambda 2$ p fragment (Fig. 1Aa), which contains the $\lambda 2$ -chain promoter, with a nuclear extract from the J558L plasmacytoma cell line, results in the formation of five complexes. These are called complexes 2.1a (previously called 2.1), 2.1b, 2.2, 2.3, and 2.4 (Fig. 1B, lane 'none'). The composition of these complexes has previously been determined (22). Complexes 2.2 and 2.4 resulted from the binding of Oct-1 and Oct-2, respectively, to the *O* motif of $\lambda 2$ p. Complex 2.3 arisen from the binding of NF- $\lambda 2$ to NF- $\lambda 2$ M, and complexes 2.1a and 2.1b are the tertiary complexes which resulted from simultaneous binding of NF- $\lambda 2$ and Oct-1 and of NF- $\lambda 2$ and

Oct-2, respectively, to the $\lambda 2$ p probe. Fig. 1B also shows that all the competitor fragments used were able to compete to about the same extent against the formation of complexes 2.1a, 2.1b, 2.2 and 2.4. This is because at least one of the components of these complexes is an octamer-binding protein, and all the competitors used contained *O*.

The intensity of complex 2.3 was unaffected when a fragment containing the κ mopc41-chain promoter (κ p) was used as a competitor (Fig. 1B). The intensity of this complex, however, decreased to various degrees when fragments containing the Hmopc315-chain promoter (Hp), the κ -chain intronic enhancer (κ e) and the H-chain intronic enhancer (He) were used as competitors (Fig. 1B). The ability to compete was, however, less than that of the $\lambda 2$ p fragment itself. Among the heterologous competitors of complex 2.3 formation, He was the most efficient one. These results suggested that Hp, κ e and He, but not κ p, contain a motif(s) with similar binding property to that of the NF- $\lambda 2$ M.

μ E3 competes for NF- $\lambda 2$ binding

To find out which region of He competes for the formation of complex 2.3, the He fragment was digested with *Pvu*II (Fig. 1Ad) to generate a 381-bp fragment containing μ E1 and part of μ E2 (E1+E2 fragment), and a 302-bp fragment containing μ E3, μ E4 and *O* (E3+E4+*O* fragment). Fig. 2A shows that of the two fragments, it is the E3+E4+*O* fragment that contained the competition ability. Removal of μ E3 from this fragment (fragment E4+*O*) completely abolished its competence (Fig. 2A). To confirm that μ E3 and its immediately surrounding sequence is

Fig. 2. Localization of an element present in the H-chain enhancer which competes for binding to NF- $\lambda 2$. $\lambda 2$ p was used as a probe in competition experiments with a nuclear extract prepared from J558L. The molar ratios of probe to competitor are indicated above the lanes. The competitor DNAs used are indicated above the brackets. Absence of competing DNA in the binding reaction is also shown (lane none). (A) Competition experiments with H-chain enhancer fragments. E1+E2 represents the 381-bp *Xba*I-*Pvu*II fragment containing μ E1 and part of μ E2 (Fig. 1d); E3+E4+*O* represents the 302-bp *Pvu*II-*Eco*RI fragment containing μ E3, μ E4 and *O* (Fig. 1d); E4+*O* represents the 51-bp *Dde*I-*Hinf*I fragment encompassing μ E4 and *O*. (B) μ E3 competes for NF- $\lambda 2$ binding. An oligonucleotide containing either μ E3 or NF- $\lambda 2$ M (see Fig. 3A for sequences), and a 76-bp *Hind*III-*Eco*RI fragment containing *O* (22) were used as competitor DNAs. (C) USF-BS competes for NF- $\lambda 2$ binding. An oligonucleotide containing the binding site for USF (see Fig. 3A for sequences) of the adenovirus major late promoter was used as competitor DNAs.

Fig. 3. (A) Sequence comparison of the oligonucleotides used as probes and competitors. The oligonucleotides are named by the motif they contain. Sequence numbering corresponds to the originally reported ones for NF- $\lambda 2$ M (22), USF-BS (29), μ E3 (2), and κ E3 (3). Nucleotides shared with those of the NF- $\lambda 2$ motif, within the palindrome (divergent arrow), are underlined. (B) Complexes formation by NF- $\lambda 2$ M, USF-BS, μ E3, and κ E3. The probes used, shown above the brackets, were incubated with a nuclear extract prepared from J558L. The absence (lane none) and presence of competitor DNAs in the binding reaction, at a probe/competitor ratio of 1:1000, are indicated above the lanes. O represents the 76 bp *Hind*III-*Eco*RI fragment containing the octamer motif (22). S and F indicate the complexes formed with the NF- $\lambda 2$ M and USF-BS probes, and U and L indicate the complexes formed with the μ E3 and E3 probes.

the element responsible for the competition for complex 2.3 formation, oligonucleotides containing separately the $\mu E3$ and the $NF-\lambda 2M$ were used as competitors (see Fig. 3A for sequences). Fig. 2B shows that the formation of all the complexes containing $NF-\lambda 2$, that is, 2.1a, 2.1b and 2.3, was prevented by both competitors with similar efficiencies. Thus, we concluded that the region involved in competition for $NF-\lambda 2$ binding includes $\mu E3$ and/or the immediately adjacent sequences. (It also shows that, as expected, the use of an *O*-containing oligonucleotide as competitor prevented complexes 2.1a, 2.1b, 2.2, and 2.4 formation).

$NF-\lambda 2$ binding to $NF-\lambda 2M$, $\mu E3$, $\kappa E3$ and $USF-BS$

It has been found that $\mu E3$ resembles a motif in the adenovirus major late promoter, to which a cell-type ubiquitous transcription factor called USF or MLTF binds (28, 29). In fact, a human proteins called TFE3, the gene of which was isolated by using the ability of this protein to bind to $\mu E3$, also binds to $USF-BS$ (30). USF and TFE3 are however different protein species (30, 31). In addition, another factor that binds to $\mu E3$, called $NF-\mu E3$ or $\mu EBP-C2$ (21, 32), also appears to be different from TFE3 (30). Since $\mu E3$ -binding proteins bind to $USF-BS$ and since $NF-\lambda 2$ interacts with the $E3$ motif, it appeared likely that $NF-\lambda 2$ would bind to $USF-BS$. In fact, $USF-BS$ contains a sequence, CACGTGAC, which is identical to a region within the $NF-\lambda 2M$ (see Fig. 3A). That $NF-\lambda 2$ is indeed able to bind to $USF-BS$ is shown in Fig. 2C. As can be seen, an oligonucleotide containing $USF-BS$ was able to compete efficiently for the formation of all of the complexes formed by $\lambda 2p$ and $NF-\lambda 2$, i.e. complexes 2.1a, 2.1b and the 2.3 doublet. Therefore, $NF-\lambda 2$ belongs to the family of factors, whose members, USF, TFE3 and $NF-\mu E3$, cross-bind to each other's specific ligands with various degrees of affinity.

Incidentally, a band migrating slightly ahead of and partly

masked by the 2.3 complex in Fig. 2C is most likely originated from the interaction of another octamer-binding protein with the *O* motif. (The same band was also observed in Fig. 2B in experiments in which oligonucleotides containing $NF-\lambda 2M$ and $\mu E3$ were used as competitors against the $\lambda 2p$ probe). This is based on the observation that a competitor containing the *O* motif always abolished this specific complex (Fig. 2B).

To investigate whether $NF-\lambda 2$ was the murine equivalent of USF, or TFE3, it was essential to corroborate the ability of the motifs, to which these factors bind, to compete against each other. A fragment containing $\kappa E3$ was also included in this experiment because of its partial sequence homology with the other motifs and because it has been shown to be a ligand for $NF-\mu E3$ or $\mu EBP-C2$ (21, 32). Fig. 3A shows the sequences of the oligonucleotides used both as probes and as competitors and Fig. 3B shows the results of the competition assays. With the exception of the *O* control, all the competitors ($NF-\lambda 2M$, $USF-BS$, $\mu E3$, and $\kappa E3$) were able to compete against the formation of complexes generated with all the probes. The patterns obtained with the $NF-\lambda 2M$ and $USF-BS$ probes are identical. It is composed of two bands; an intense fast-migrating one (F) and a much weaker slower-migrating one (S). This band pattern differs from that generated by the $\mu E3$ and $\kappa E3$ probes, which is composed of two closely migrating complexes (upper (U) and lower (L)). The difference between these two band patterns could be due to the formation of two structurally distinct series of complexes; one with $NF-\lambda 2M$ and $USF-BS$ and another with $\mu E3$ and $\kappa E3$. Since the specific activities of the probes were fairly comparable, it is apparent that factor binding to $\mu E3$ and $\kappa E3$ probes is weaker than to $NF-\lambda 2M$ and $USF-BS$ probes (the exposure time for the $\mu E3$ and $\kappa E3$ probes was double that for the $NF-\lambda 2M$ and $USF-BS$ probes). Alternatively, the differences in patterns and band intensities may imply that some of the factors that bind to $\mu E3$

Fig. 4. Interaction of $NF-\lambda 2$ -specific complexes with anti-USF serum. Electrophoretic mobility shift assays were used to test whether $NF-\lambda 2$ is antigenically related to USF. All the antibodies used were diluted 1000 to 2500 times. (A) Reaction with antibody against full length USF. The probes used, indicated above the brackets, were incubated in the presence of anti-USF serum (lane immune), or non-immuned serum (lane naive). 'None' indicated the absence of any serum in the binding reaction. S and F indicate the complexes formed with the $NF-\lambda 2M$ and $USF-BS$ probes, and U and L indicate complexes formed with the $\mu E3$ and $\kappa E3$ probes. (B) $NF-\lambda 2$ interacts with antibodies raised against different USF domains. The numbers above the lanes correspond to the antibody raised against different domains of USF (31); i.e. 1–310, antibody raised against the full length USF; 18–105, antibody raised against amino acid residues no. 18–105; 106–196, antibody raised against amino acid residues no. 106 to 196; and 274–310, antibody raised against amino acid residues no. 274 to 310. Reactions were carried out in the presence of anti-USF (lane immune), or non-immuned serum (lane naive). (C) Anti-USF antibody does not cross react with TFE3. $USF-BS$ was used as a probe with USF and TFE3 synthesized in an *in vitro* transcription and translation system (indicated above the brackets). 'Mock' refers to an *in vitro* translation reaction performed in the absence of exogenous mRNA.

and $\kappa E3$ are different from those that bind to *NF- $\lambda 2M$* and *USF-BS*. Nevertheless, these results suggest that the factors that bind to *NF- $\lambda 2M$* and *USF-BS* are the same and that the sequences of these two ligands (the sequence recognized by the factor(s)) are identical.

Antigenic similarity between *NF- $\lambda 2$* and USF

To further determine whether the factor recognizing *NF- $\lambda 2M$* , *USF-BS* and $\mu E3$ are related, a rabbit polyclonal antiserum against the 43 kD USF, purified from bacterial extract, was used (27). This antiserum abolished the doublet of complexes formed by *USF-BS* and USF synthesized by an *in vitro* transcription-translation system, or by expression of its gene in bacteria (unpublished data). Figs. 4Ab and 4Ac show that the complexes formed by *NF- $\lambda 2M$* and *USF-BS* were destroyed by the anti-USF serum. Addition of USF-specific serum (but not 'naive' serum) to a binding reaction with the $\lambda 2p$ probe resulted in removal of complexes 2.1a; 2.1b, and 2.3 (Fig. 4Aa). Since these three complexes are precisely the ones that contain *NF- $\lambda 2$* , these results suggest that anti-USF serum binds specifically to *NF- $\lambda 2$* . Of the two complexes formed by the $\mu E3$ and $\kappa E3$ probes, the upper complex was specifically abolished by the anti-USF serum whereas the lower complex remained intact (Fig. 4Ad and 4Ae). Therefore, the lower complex, which is not antigenically related to USF, must be generated by binding of *E3*-related proteins whereas the upper complex resulted from binding of the *E3* motifs to a factor with epitopes common to USF. In fact, recent evidence suggests that the major component (43 kD) of the purified $\mu E3$ binding proteins (32) is probably the same as USF on the basis

of cross reactivity with anti-USF antibody (personal communication, C. Cooper and K. Calame).

The antiserum used in the experiments above was raised against the full length polypeptide chain of USF. The same type of experiments were performed with three additional antibodies which were raised against specific domains of USF (i.e. polypeptide chains containing amino acid residues 18–105, 106–196 and 274–310, respectively (27)). As shown in Fig. 4B, all three antibodies abolished the complexes generated from binding of *NF- $\lambda 2$* to the probe; i.e. complexes 2.1a, 2.1b, and 2.3. Similar results were also obtained with these antibodies using *USF-BS* and *E3* as probes (unpublished data). Thus, the epitope similarity between USF and *NF- $\lambda 2$* extends to specific domain of the polypeptide chain.

Since both TFE3 and USF appear to recognize a spectrum of *E3*-like motifs, it was possible that these proteins shared epitopes. This was tested by using the disruptive effect of the antiserum mentioned above on complex formation with the oligonucleotide containing a USF binding site (31) in the standard antibody/gel shift assay. Fig. 4C shows that, in the presence of *in vitro* synthesized USF, anti-USF antibody specifically destroyed the complex; whereas in the presence of TFE3, also synthesized by an *in vitro* transcription-translation system, the complex remained intact. Furthermore, anti-USF antibody specifically removed the USF-complex and left the TFE3 complex intact when USF and TFE3 were present in the same reaction. (The lanes labelled 'mock' are controls which show that the rabbit reticulocyte lysate used for the *in vitro* translation lacked proteins able to interact specifically with the probe (Fig. 4C)). These results show that TFE3 and USF are antigenetically unrelated.

Exogenous USF stimulates transcription from $\lambda 2$ -chain promoter

We have previously shown that complex 2.3 is generated by the binding of *NF- $\lambda 2$* to the *NF- $\lambda 2M$* and that such an interaction is important for the regulation of $\lambda 2$ -chain gene expression *in vivo* (22). Since the information accumulated so far strongly suggested that *NF- $\lambda 2$* is the murine counterpart of USF, we tested the ability of USF to stimulate *in vitro* transcription off the $\lambda 2$ promoter. In this experiment, instead of J558L, Namalwa cells were used as the source of nuclear extract due to their excellent ability to carry out transcription activity *in vitro*. A plasmid called p $\lambda 2$ containing the rearranged $\lambda 2$ -chain gene (9) was used as a template and transcribed either in a control (flow through of the preimmune anti-USF-IgG column) or USF-depleted Namalwa extract (flow through of the immune anti-USF IgG column). Although the band intensity was much weaker than the one generated with the control template (unpublished data), transcription off the p $\lambda 2$ template generated a 92-nt protected fragment, which corresponds to the authentic start site (9) (Fig 5A, lane 1). Transcription from the $\lambda 2$ -chain promoter in USF-depleted extract was four- to sixfold less efficient than that from the control extract (lane 2); and addition of exogenous USF restored transcription activity (lane 3). Incidentally, the amount of USF added to the depleted extract was about 5 fold higher than that originally present in the extract, and this could account for the slight increase in the intensity of the same band. As well, a longer transcript initiated from a pseudo TATA box located immediately upstream of the *O* site could also be due to the presence of excess amount of exogenous USF (lane 3). Similar results were obtained with a template containing the $\lambda 2$ -chain promoter linked to the CAT gene vector (unpublished data).

Fig. 5. USF stimulates transcription off the $\lambda 2$ -chain promoter in an *in vitro* transcription assay. *In vitro* transcription assays were performed with supercoiled plasmid p $\lambda 2$ and Namalwa nuclear extracts. The extract used was either control (lane 1), USF depleted (lane 2) or USF depleted and supplemented with bacterially expressed purified USF (lane 3). The protected fragment corresponding to the authentic start site of the $\lambda 2$ -chain transcript is indicated as a thick arrow; the fragment corresponding to a nonspecific initiation of the transcript is indicated as a thin arrow. The numbers represent length, in nucleotides, of DNA size markers.

These experiments clearly demonstrated that USF stimulates transcription off the $\lambda 2$ promoter *in vitro*.

There are two major forms of the USF protein which can be distinguished both by their slightly different mobilities in sodium dodecyl sulfate gel electrophoresis (apparent molecular weights 44 and 43 kD, respectively) and by the different electrophoretic mobilities of the corresponding protein-DNA complexes (33). Likewise the complex that NF- $\lambda 2$ forms with $\lambda 2p$ (complex 2.3) is also a doublet composed of two closely migrating bands. Therefore, USF and NF- $\lambda 2$ share a) the property to generate a doublet of complexes with their ligands, b) the sequence of their ligands, c) epitope specificity, and d) stimulatory effect on the $\lambda 2$ -chain promoter. Thus, we conclude that NF- $\lambda 2$ is the murine homologue of human USF.

It has been observed that human and amphibian USF species have a high degree of sequence conservation (>80%) (34). This observation and the cross reactivity of NF- $\lambda 2$ to antisera against specific domains of USF suggests that both proteins share long regions of sequence homology. A definite proof of this could only be obtained by cloning and sequencing the NF- $\lambda 2$ gene.

USF, TFE3 and TFEB (another human protein that interacts with the MLTF binding site (35)) are different proteins, yet they seem to recognize a common binding site, i.e. CACGTG. They are all members of the *c-Myc*-related family of DNA-binding proteins (36), the carboxyl termini of which are characterized by a basic region (b), a helix-loop-helix motif (HLH), and a leucine zipper repeat (Zip) (30, 31). Interestingly, association of a newly identified protein called *Max*, which also belongs to the family of b-HLH-Zip proteins, with either *c-Myc*, *N-Myc*, or *L-Myc* forms a heterodimer that binds to CACGTG; whereas under the same conditions, neither of the individual proteins are able to bind (37). *In vitro*, USF does not form heterodimers readily with *c-*, *N-*, and *L-Myc* (37), nor does it with several other proteins containing Zip (*c-jun*) or HLH (*MyoD*, myogenin, and *c-Myc*) motifs (31).

It has been shown that USF and the TATA-binding transcription factor TFIID interact cooperatively when simultaneously bound to the adenovirus major late promoter (29). Based on the similarity between USF and NF- $\lambda 2$ reported here, we would predict that NF- $\lambda 2$ interacts in the same way with TFIID.

It will be of interest to know whether TFE3, or other *E3* binding proteins, has a similar stimulatory effect on $\lambda 2$ -chain gene expression. However, the binding activity of TFE3 is not readily detectable in a crude nuclear extract unless the extract has been denatured and renatured in a southwestern analysis (30). This presents a problem at this stage because all the experiments described previously were performed with crude nuclear extracts. Based on the fact that TFE3 stimulates transcription from an artificial promoter consisting of multiple copies of the *E3* motif linked to a TATA box (30), we suspect that it may also play some role in stimulating transcription off the $\lambda 2$ chain promoter. A definite answer can be obtained when further information regarding the behaviour of TFE3 in a crude extract becomes available.

Finally, although we have demonstrated that the interaction of the murine counterpart of USF, (or an antigenically-related USF), with the *E3*-like site (NF- $\lambda 2M$) of the $\lambda 2$ promoter is crucial for the expression of the $\lambda 2$ -chain gene, the precise role of the *E3*-binding proteins (i.e. USF, TFE3, TFEB and others) for transcription activation via the *E3*-binding site *in vivo* is still not clear. However, the ability of proteins similar to the

E3-binding factors to form polymeric complexes suggests that their affinity to the target DNA is modulated by specific protein-protein interactions. Hence, it is likely that interactions between transcription factors play a major role in the regulation of immunoglobulin gene expression.

ACKNOWLEDGMENTS

We thank Dr. Polly Gregor of The Rockefeller University for hospitality, and helpful discussions. We also like to thank Dimitra E. Tzamtzis for technical assistance in the construction of pUC19He. This work was supported by a grant from the National Cancer Institute of Canada to H.M., and by U.S. Public Health Service Grant CA42567 to R.G.R.. P.P. was supported by an EMBO postdoctoral fellowship.

REFERENCES

- Banerji, J., Olson, L. and Schaffner W. (1983) *Cell*, **33**, 729–740.
- Gillies, S. D., Morrison, S. L., Oi, V. T. and Tonegawa, S. (1983) *Cell*, **33**, 717–728.
- Picard, D. and Schaffner, W. (1984) *Nature*, **307**, 80–82.
- Queen, C. and Baltimore D. (1983) *Cell*, **33**, 741–748.
- Queen, C. and Stafford, J. (1984) *Mol. Cell. Biol.*, **4**, 1042–1049.
- Meyer, K.B. and Neuberger, M. S. (1989) *EMBO J.*, **8**, 1959–1964.
- Pettersson, S., Cook, G. P., Bruggemann, M., Williams, G. T. and Neuberger, M.S. (1990) *Nature (London)*, **344**, 165–168.
- Hagman, J., Rudin, C.M., Haasch, D., Chaplin, D. and Storb, U. (1990) *Genes Dev.*, **4**, 978–992.
- Alonso, A., Chang, L. A. and Murialdo H. (1990) *Mol. Immunol.*, **27**, 115–127.
- Church, G.M., Ephrussi, A., Gilbert, W. and Tonegawa, S. (1985) *Nature (London)*, **313**, 798–801.
- Ephrussi, A., Church, G. M., Tonegawa, S. and Gilbert, W. (1985) *Science*, **227**, 134–140.
- Falkner, F. G. and Zachau, H. G. (1984) *Nature (London)*, **310**, 71–74.
- Lenardo, M., Pierce, J. W. and Baltimore, D. (1987) *Science*, **236**, 1573–1577.
- Parslow, T. G., Blair, D. L., Murphy, W. J. and Granner, D. K. (1984) *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 2650–2654.
- Landolfi, N. F., Capra, J. D. and Tucker, P. W. (1986) *Nature (London)*, **323**, 548–551.
- Singh, H., Sen, R., Baltimore, D. and Sharp, P. A. (1986) *Nature (London)*, **319**, 154–158.
- Staudt, L.M., Singh, H., Sen, R., Wirth, T., Sharp, P. A. and Baltimore, D. (1986) *Nature (London)*, **323**, 640–643.
- Currie, R. A. and Roeder, R. G. (1989) *Mol. Cell. Biol.*, **9**, 4239–4247.
- Nelms, K. and Van Ness, B. (1990) *Mol. Cell. Biol.*, **10**, 3843–3846.
- Peterson, C. L., Orth, K. and Calame, K. L. (1986) *Mol. Cell. Biol.*, **6**, 4168–4178.
- Sen, R. and Baltimore, D. (1986) *Cell*, **46**, 705–716.
- Chang, L. A. and Murialdo, H. (1990) *Mol. Cell. Biol.*, **10**, 5894–5902.
- Seidman, J. G., Max, E.E. and Leder, P. (1979) *Nature (London)*, **280**, 370–375.
- Rinfret, A., Horne, C., Dorrington, K. J. and Klein, M. (1989) *Mol. Immunol.*, **26**, 431–434.
- Yu, H., Porton, B., Shen, L. and Eckhardt, L. A. (1989) *Cell*, **58**, 441–448.
- Sambrook, J., Fritsch, E. F. and Maniatis, T. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor University Press, Cold Spring Harbor.
- Pognonec, P. and Roeder, R. G. (1991) *Mol. Cell. Biol.*, **11**, 5125–5136.
- Carthew, R. W., Chodosh, L. A. and Sharp, P. A. (1985) *Cell*, **43**, 439–448.
- Sawadogo, M. and Roeder, R. G. (1985) *Cell*, **43**, 165–175.
- Beckmann, H., Su, L. K. and Kadesch, T. (1990) *Genes Dev.*, **4**, 167–179.
- Gregor, P. D., Sawadogo, M. and Roeder, R. G. (1990) *Genes Dev.*, **4**, 1730–1740.
- Peterson, C. L. and Calame, K. (1989) *Mol. Cell. Biol.*, **9**, 776–786.

33. Sawadogo, M., Van Dyke, M. W., Gregor, P. D. and Roeder, R. G. (1988) *J. Biol. Chem.*, **263**, 11985–11993.
34. Kaulen, H., Pognonec, P., Gregor, P. D. and Roeder, R. G. (1991) *Mol. Cell. Biol.*, **11**, 412–424.
35. Carr, C. S. and Sharp, P. A. (1990) *Mol. Cell. Biol.*, **10**, 4384–4388.
36. Murre, C., McCaw, P. S. and Baltimore, D. (1989) *Cell*, **56**, 777–783.
37. Blackwood, E. M. and Eisenman, R. N. (1991) *Science*, **251**, 1211–1217.
38. Grosschedl, R. and Baltimore, D. (1985) *Cell*, **41**, 885–897.
39. Mason, J. O., Williams, G. T. and Neuberger, M. S. (1985) *Cell*, **41**, 479–487.