Milanez, S., & Mural, R. J. (1988) *Gene 66*, 55-63.
Miziorko, H. M., Brodt, C. A., & Krieger, T. J. (1990) *J. Biol. Chem. 265*, 3642-3647.
Möller, W., & Amons, R. (1985) *FEBS Lett. 186*, 1-7.
Muller, E. D., Chory, J., & Kaplan, S. (1985) *J. Bacteriol. 161*, 469-472.
Nagata, S., Tanizawa, K., Escki, N., Sakamoto, Y., Oshima, T., Tanaka, H., & Soda, K. (1988) *Biochemistry 27*, 9056-9062.
Porter, M. A., Stringer, C. D., & Hartman, F. C. (1988) *J. Biol. Chem. 263*, 123-129.
Quivey, R. J., Jr., & Tabita, F. R. (1984) *Gene 31*, 91-101.
Raines, C. A., Lloyd, J. C., Longstaff, M., Bradley, D., & Dyer, T. (1988) *Nucleic Acids Res. 16*, 7931-7942.
Rittenhouse, J., Harrsch, P. B., Kim, J. N., & Marcus, F. (1986) *J. Biol. Chem. 261*, 3939-3953.
Roesler, K. R., & Ogren, W. L. (1988) *Nucleic Acids Res. 16*, 7192.
Rogers, D. T., Hiller, E., Mitsock, L., & Orr, E. (1988) *J. Biol. Chem. 263*, 6051-6057.
Ronson, C. W., Nixon, B. T., & Ausubel, F. M. (1988) *Cell 49*, 578-581.
Scheffler, J. E., & Fromm, H. J. (1986) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 45*, 1868.
Siebert, K., Schobert, P., Bowien, B. (1981) *Biochim. Biophys. Acta 658*, 35-94.
Springgate, C. F., & Stachow, C. S. (1972) *Arch. Biochem. Biophys. 152*, 13-19.
Suwanto, A., & Kaplan, S. (1989) *J. Bacteriol. 171*, 5850-5859.
Tabita, F. R. (1980) *J. Bacteriol. 143*, 1275-1280.
Tabita, F. R. (1988) *Microbiol. Rev. 52*, 155-189.
Tabita, F. R., Gibson, J. L., Falcone, D. L., Lee, B., & Chen, J.-H. (1990) *FEMS Microbiol. Rev.* (in press).
Tanabe, T., Nukada, T., Nishikawa, Y., Sugimoto, K., Suzuki, H., Takahashi, H., Noda, M., Haga, T., Ichiyama, A., Kangawa, K., Minamino, N., Matsuo, H., & Numa, S. (1985) *Nature 315*, 242-245.
Trüper, H. G., & Pfennig, N. (1978) in *The Photosynthetic Bacteria* (Clayton, R. K., & Sistrom, W. R., Eds.) pp 19-27, Plenum, New York.
Xu, G.-J., Natalini, P., Suda, H., Tsolas, O., Dzugaj, A., Sun, S. C., Pontremoli, S., & Horecker, B. L. (1982) *Arch. Biochem. Biophys. 214*, 688-694.
Yanisch-Perron, C., Vieira, J., & Messing, J. (1985) *Gene 33*, 103-119.
Zhou, C., Hwang, S.-H., & Jong, A. Y. (1989) *J. Biol. Chem. 264*, 9022-9029.

Human IgE-Binding Protein: A Soluble Lectin Exhibiting a Highly Conserved Interspecies Sequence and Differential Recognition of IgE Glycoforms[†,‡]

Michael W. Robertson, Keith Albrandt, Daryle Keller, and Fu-Tong Liu*
Medical Biology Institute, Division of Molecular Biology, 11077 North Torrey Pines Road, La Jolla, California 92037
Received February 27, 1990; Revised Manuscript Received May 23, 1990

ABSTRACT: IgE-binding protein (εBP) refers to a protein originally identified in rat basophilic leukemia cells by virtue of its affinity for IgE. It is now known to be a β-galactoside-binding lectin equivalent to carbohydrate-binding protein 35 (CBP 35). More recently, its identity to Mac-2, a macrophage cell-surface protein, has been established. cDNA coding for human εBP has been cloned from a human HeLa cell cDNA library and contains an open reading frame of 750 base pairs encoding a 250 amino acid protein. Like the rat and murine counterparts, the human εBP amino acid sequence can be divided into two domains with the amino-terminal domain consisting of a highly conserved repetitive sequence (YPGXXXPGA) and the carboxyl-terminal domain containing sequences shared by other S-type lectins. The human εBP sequence exhibits extensive homology to murine and rat εBP with 84% and 82% identity, respectively. The homology is particularly striking in the carboxyl-terminal domain where 95% identity is found between human and murine sequences in a stretch of over 70 amino acids. A survey of εBP mRNA expression from several lymphocyte cell lines revealed that the level of εBP transcription may reflect a relationship between cell differentiation and εBP expression. Finally, human εBP was purified from several human cell lines and shown to possess lactose-binding characteristics and cross-species reactivity to murine IgE. Surprisingly, three different human myeloma IgE proteins did not show reactivity to human εBP. However, after neuraminidase treatment of each human IgE, pronounced binding to εBP was observed, thereby indicating that only specific IgE glycoforms can be recognized by εBP.

IgE-binding protein (εBP)[1] (Liu, 1990) refers to a M_r 31 000 protein with IgE-binding activity, originally identified in rat basophilic leukemia (RBL) cells (Liu & Orida, 1984; Liu et al., 1985). Cloning and sequencing of cDNA revealed a novel sequence with several interesting structural features (Liu et al., 1985; Albrandt et al., 1987). The protein is composed of 2 domains: the amino-terminal domain contains tandem repeats of a highly conserved sequence of 9 amino acids [Tyr-Pro-Gly-(Pro/Gln)-(Ala/Thr)-(Pro/Ala)-Pro-Gly-Ala]; the

[†] This work was supported by National Institutes of Health Grants AI-19747 and AI-20958 to F.-T.L.
[‡] The nucleic acid sequence in this paper has been submitted to GenBank under Accession Number J02921.
* To whom correspondence should be addressed at the Department of Molecular and Experimental Medicine, Research Institute of Scripps Clinic, 10666 N. Torrey Pines Rd., La Jolla, CA 92037.

[1] Abbreviations: εBP, IgE-binding protein; RBL, rat basophilic leukemia; CBP 35, carbohydrate-binding protein 35; RL-29, rat lung lectin 29; HL-29, human lung lectin 29; hnRNP, heterogeneous nuclear ribonucleoprotein; Mac-2, murine macrophage cell-surface protein 2.

0006-2960/90/0429-8093$02.50/0 © 1990 American Chemical Society

carboxyl-terminal domain contains 21 of the 22 basic amino acids found in this protein (Albrandt et al., 1987). A subsequent study revealed that the protein has wide tissue distribution (Gritzmacher et al., 1988), suggesting that its function may not be limited to recognition of IgE. The primary cellular localization of this protein within the cytoplasm has also been demonstrated (Gritzmacher et al., 1988).

Although εBP contains interesting structral features, the function of this protein remains undefined. A recent development shed significant light on our understanding of the biochemical properties of εBP and provided a basis for probing physiological roles of this protein: another group of researchers, working with a carbohydrate-binding protein, CBP 35 (Roff & Wang, 1983), cloned cDNA coding for this protein (Jia & Wang, 1988) which revealed that it is highly homologous to εBP. In a collaborative effort, we showed that εBP, like CBP 35, has an intrinsic galactose-binding activity and that εBP and CBP 35 are rat and mouse protein homologues (Laing et al., 1989). Subsequently, it became clear that two other groups have been working with a similar lectin. One group has designated the lectin L-34, and the deduced protein sequence from their cDNA showed significant sequence homology with rat εBP (Raz et al., 1989). Another group has designated the lectin RL-29 (Leffler & Barondes, 1986) and HL-29 (Sparrow et al., 1987), from rat and human, respectively, and also has sequence data indicative of a close relationship with εBP (Leffler et al., 1989). It is now clear that εBP is an endogenous soluble lectin (Barondes, 1988) with S-type carbohydrate-recognition activity (Drickamer, 1988).

The exact physiological role(s) of εBP is (are) presently unknown, although studies of similar lectins in other laboratories have revealed interesting characteristics. CBP 35 can be found in the nucleus and may be a component of the heterogeneous nuclear ribonucleoprotein complex (hnRNP) (Laing & Wang, 1988); the overall level of CBP 35, as well as the amount of this protein in the nucleus, has been found to increase dramatically in proliferating 3T3 fibroblasts (Moutsatsos et al., 1987; Agrwal et al., 1989). These results suggest that this lectin may be a component of a growth-regulating system. Studies by Raz and co-workers related the expression of L-34 on the cell surface to neoplastic transformation and to the metastatic potential of tumor cells (Raz & Lotan, 1987). Recently, cDNA coding for the Mac-2 antigen, a murine macrophage cell-surface protein, has been shown to be identical with CBP 35, and thus a homologue of rat εBP (Cherayil et al., 1989). Several possible biological functions of this protein in macrophages, including a likely role as a cell-surface receptor involved in lectin-mediated phagocytosis, may provide a clue to the function of this protein in other biological systems.

In order to extend the study of this lectin to the human system, we decided to clone cDNA coding for the human counterpart of εBP. Here, we report the nucleotide sequence of human εBP cDNA. Comparison of the deduced amino acid sequence with that of the rat and murine sequences indicates that the proteins are highly conserved. Northern blot analysis identified a 1.1-kb mRNA similar in size to the previously characterized mRNA for rat εBP. Further, we show that εBP expression is cell-type-specific among various lymphoid cell lines. Protein of M_r 30000 could be isolated from several εBP-positive human cell lines with lactosyl-Sepharose and murine IgE–Sepharose and eluted specifically with lactose. In addition, an interesting pattern of εBP reactivity to human myeloma IgE was found, suggesting that only specific IgE glycoforms are recognized by human εBP.

Materials and Methods

Cell Lines and Reagents. The following cell lines were employed for this study: human B-lymphocyte cell line Wil-2 (from J. Marcelletti of this institute), human B-cell line RPMI 8866 (from D. Katz of this institute), human monocyte-like cell line U937 (American Type Cell Culture, CRL1593, Rockville, MD), rat basophilic leukemia (RBL) cell line (from H. Metzger, National Institutes of Health, Bethesda, MD), and Swiss 3T3 fibroblasts (American Type Cell Culture CCL92). Murine or human IgE–Sepharose 4B was prepared by using CNBr-activated Sepharose 4B (Pharmacia, Piscataway, NJ) and murine monoclonal anti-DNP IgE (Liu et al., 1980) or human myeloma IgE PS (from K. Ishizaka, La Jolla Institute of Allergy and Immunology, La Jolla, CA); DZA and BEDORA (from H. Spiegelberg, Scripps Clinic, La Jolla, CA) were prepared by following the manufacturer's protocol. Lactosyl-Sepharose 4B was prepared by derivatizing Sepharose 4B with lactose using a previously reported procedure (Levi & Teichberg, 1981). Rabbit antiserum directed to a synthetic peptide (FNPRFNENNRRVIVC), designated anti-εBP1, was employed in immunoblotting studies as described previously (Gritzmacher et al., 1988).

Screening cDNA Clones. cDNA clones were isolated from either a normal human lung λgt11 cDNA library (Clontech, Palo Alto, CA) or a HeLa cell library containing cDNA inserts in the λ Zap II vector (Stratagene, La Jolla, CA). Both libraries contain cDNA inserts cloned into the *Eco*RI site. The human lung λgt11 cDNA library (9×10^5 plaques) was screened by colony hybridization (Hanahan & Meselson, 1980) using nick-translated cDNA probes derived from the rat εBP cDNA clone E8 (Albrandt et al., 1987) and consisting of a 370 bp *Xho*I–*Sph*I amino-terminal cDNA fragment and a 378 bp *Sph*I–*Hae*III carboxy-terminal cDNA fragment. After identification of positive plaques, DNA from individual λgt11 clones was prepared followed by *Eco*RI digestion, isolation of the εBP insert, and subcloning into pUC19. One of the clones, 27.1, is described in this report.

The HeLa cell λ Zap II library (5×10^5 plaques) was initially screened with a ^{32}P-end-labeled 30-mer oligonucleotide probe (5′-AGGCCATCCTGGAGGGTTTGGGTTTCC-AGA-3′; Genetic Designs Inc., Houston, TX) representing the reverse complement of a sequence near the 5′ end of the human εBP-coding region (and corresponding to amino acids 14–23 of rat εBP) determined from a cDNA clone (27.1) identified in the human lung cDNA library described above. Hybridization was performed as described previously (Liu et al., 1988), and 67 positive plaques were identified. Selected plaques were then treated with R408 helper-phage to excise the pBluescript SK(−) phagemid following the manufacturer's protocol. By subsequent restriction endonuclease analysis, four clones containing the longest cDNA inserts were selected for DNA sequencing, and one clone (2.2) is included in this report.

Nucleotide Sequencing. Nested deletion subclones were produced for all cDNA clones by treatment of the plasmid DNA with *Exo*III–mung bean nuclease, ligation, and transformation, following the manufacturer's protocol (Stratagene). DNA was sequenced on both strands by the dideoxy chain termination method (Sanger et al., 1977) using modified phage T7 DNA polymerase (Sequenase, United States Biochemical Corp., Cleveland, OH).

In Vitro Transcription and Translation. The clone 2.2 cDNA was digested with *Pvu*II, which produced a 1387 bp fragment containing the entire cDNA sequence and the T7 RNA polymerase promoter. The DNA was used to synthesize capped mRNA in vitro using T7 RNA polymerase (Strata-

gene) and a commercial transcription kit and protocol (Promega Corp., Madison, WI). The mRNA was then translated in vitro using [^{35}S]methionine in a rabbit reticulocyte lysate system (Promega). The εBP translation product was affinity-purified and analyzed as described below.

RNA Blot Analysis. Isolation of poly(A+) RNA from various cell lines, formaldehyde–agarose gel electrophoresis, and subsequent blotting to nitrocellulose filters were performed by using standard procedures (Maniatis et al., 1982). Hybridization was performed at 42 °C in 50% formamide/0.04% Ficoll/0.04% poly(vinylpyrrolidone)/0.04% BSA/75 mM NaCl/25 mM PIPES (pH 6.8)/25 mM EDTA/0.2% SDS/100 μg mL^{-1} tRNA/100 μg mL^{-1} denatured salmon sperm DNA using a nick-translated cDNA probe consisting of a 560 bp εBP fragment (derived from nested deletion of clone 27.1 and corresponding to clone 2.2 sequence spanning nucleotides 203–762) in pUC19. A final blot wash was performed at 37 °C in 0.1% SDS/30 mM NaCl/3 mM sodium citrate, pH 7.0, prior to autoradiography.

Immunoblot Analysis. Cell lysates were prepared by using Triton X-100 following a previously described procedure (Liu & Orida, 1984). Affinity purification was performed by mixing cell lysate supernatant with murine IgE–Sepharose 4B, human myeloma IgE–Sepharose 4B, or lactosyl-Sepharose 4B for 3 h at 4 °C followed by extensive washing with 1% Triton X-100/10 mM Tris-HCl (pH 7.5)/5 mM EDTA/150 mM NaCl followed by 62.5 mM Tris-HCl, pH 6.8. Bound proteins were eluted with either 0.2 M lactose or 2% SDS, fractionated by SDS–PAGE (Laemmli, 1970), transferred to filters (Immobilon, Millipore, Bedford, MA), and immunoblotted with a polyclonal anti-peptide antibody (anti-εBP1) as described (Gritzmacher et al., 1988).

Neuraminidase Treatment of Human Myeloma IgE–Sepharose and Reactivity with Rat ^{125}I-εBP. Human myeloma IgE (PS, DZA, or BEDORA)–Sepharose beads were suspended in PBS and treated with 0.5 unit of neuraminidase (Sigma, St. Louis, MO) for 1 h at 4 °C. Subsequently, the beads were washed several times with PBS and then used for immunoblot experiments or in a binding assay using radioiodinated recombinant rat εBP (L. Frigeri, M.W.R., and F.-T. L., unpublished results). The latter experiment utilized 25 μL of neuraminidase-treated beads suspended in 400 μL of 1% Triton-X100/10 mM Tris-HCl pH 7.5)/5 mM EDTA/150 mM NaCl and ^{125}I recombinant rat εBP labeled by the Chloramine T method (McDonahey & Dixon, 1966). After incubation for 3 h at 4 °C, the beads were washed extensively as described above, and bound protein was eluted with 50-μL portions of 0.2 M lactose. Aliquots of the pooled lactose eluate were characterized by SDS–PAGE followed by autoradiography.

Results

Cloning of Human εBP cDNA. As we had previously found that rat εBP is abundantly expressed in the lung, we first screened a normal human lung cDNA library with a rat εBP cDNA (Albrandt et al., 1987) probe and identified several clones. The clone (27.1) with the longest insert (2474 bp) was completely sequenced. This sequence was found to be highly homologous to rat εBP cDNA, with the following exceptions: (i) there is a much longer 5′-untranslated sequence in clone 27.1; (ii) there is a single base insertion in the coding region of clone 27.1, resulting in an interruption of the open reading frame. A computer data base search then revealed that a part of the "5′-untranslated sequence" matches closely with the coding region of the ferritin gene. We thus concluded that clone 27.1 is a fused cDNA that also has a single base insertion, probably results of cloning artifacts. Several other clones were found to contain partial εBP coding sequence. Significantly, each of these clones did not have the single base mutation noted in clone 27.1.

```
CCAGCCAACGAGCGGAAA ATG GCA GAC AAT TTT TCG CTC CAT GAT GCG TTA     51
                    M   A   D   N   F   S   L   H   D   A   L
                                                        10
TCT GGG TCT GGA AAC CCA AAC CCT CAA GGA TGG CCT GGC GCA TGG GGG     99
 S   G   S   G   N   P   N   P   Q   G   W   P   G   A   W   G
                                 20
AAC CAG CCT GCT GGG GCA GGG GGC TAC CCA GGG GCT TCC TAT CCT GGG    147
 N   Q   P   A   G   A   G   G   Y   P   G   A   S   Y   P   G
         30                                      40
GCC TAC CCC GGG CAG GCA CCC CCA GGG GCT TAT CCT GGA CAG GCA CCT    195
 A   Y   P   G   Q   A   P   P   G   A   Y   P   G   Q   A   P
                             50
CCA GGC GCC TAC CAT GGA GCA CCT GGA GCT TAT CCC GGA GCA CCT GCA    243
 P   G   A   Y   H   G   A   P   G   A   Y   P   G   A   P   A
 60                                          70
CCT GGA GTC TAC CCA GGG CCA CCC AGC GGC CCT GGG GCC TAC CCA TCT    291
 P   G   V   Y   P   G   P   P   S   G   P   G   A   Y   P   S
                 80                                          90
TCT GGA CAG CCA AGT GCC CCC GGA GCC TAC CCT GCC ACT GGC CCC TAT    339
 S   G   Q   P   S   A   P   G   A   Y   P   A   T   G   P   Y
                                 100
GGC GCC CCT GCT GGG CCA CTG ATT GTG CCT TAT AAC CTG CCT TTG CCT    387
 G   A   P   A   G   P   L   I   V   P   Y   N   L   P   L   P
         110                                     120
GGG GGA GTG GTG CCT CGC ATG CTG ATA ACA ATT CTG GGC ACG GTG AAG    435
 G   G   V   V   P   R   M   L   I   T   I   L   G   T   V   K
                         130
CCC AAT GCA AAC AGA ATT GCT TTA GAT TTC CAA AGA GGG AAT GAT GTT    483
 P   N   A   N   R   I   A   L   D   F   Q   R   G   N   D   V
140                                         150
GCC TTC CAC TTT AAC CCA CGC TTC AAT GAG AAC AAC AGG AGA GTC ATT    531
 A   F   H   F   N   P   R   F   N   E   N   N   R   R   V   I
                 160                                     170
GTT TGC AAT ACA AAG CTG GAT AAT AAC TGG GGA AGG GAA GAA AGA CAG    579
 V   C   N   T   K   L   D   N   N   W   G   R   E   E   R   Q
                                 180
TCG GTT TTC CCA TTT GAA AGT GGG AAA CCA TTC AAA ATA CAA GTA CTG    627
 S   V   F   P   F   E   S   G   K   P   F   K   I   Q   V   L
         190                                     200
GTT GAA CCT GAC CAC TTC AAG GTT GCA GTG AAT GAT GCT CAC TTG TTG    675
 V   E   P   D   H   F   K   V   A   V   N   D   A   H   L   L
                         210
CAG TAC AAT CAT CGG GTT AAA AAA CTC AAT GAA ATC AGC AAA CTG GGA    723
 Q   Y   N   H   R   V   K   K   L   N   E   I   S   K   L   G
220                                         230
ATT TCT GGT GAC ATA GAC CTC ACC AGT GCT TCA TAT ACC ATG ATA TAA    771
 I   S   G   D   I   D   L   T   S   A   S   Y   T   M   I   *
                 240                                     250
TCTGAAAGGGGCAGATTAAAAAAAAAAAAAAAAGAATCTAAACCTTACATGTGTAAAGGTTTCA  834
TGTTCACTGTGAGTGAAAATTTTTACATTCATCAATATCCCTCTTGTAAGTCATCTACTTAAT   897
AAATATTACAGTGAAAG                                                  914
```

FIGURE 1: Nucleotide sequence of human εBP 2.2 cDNA and its deduced amino acid sequence. The deoxynucleotide numbering scheme is shown on the right margin, and the amino acid sequence is numbered below each line. The polyadenylation signal is underlined. The potential N-linked glycosylation site (*Asn*XaaSer/Thr) is circled. The identity between clone 27.1 and clone 2.2 begins at nucleotide 36 and amino acid 7 (leucine) in clone 2.2. The single nucleotide addition (dC) in clone 27.1 appears between nucleotides 500 and 501 in clone 2.2. In addition, nucleotide 286 (dC) in clone 2.2 is different from the corresponding position in clone 27.1 (dT), resulting in a proline (2.2) to serine (27.1) substitution.

A survey of several cell lines revealed that HeLa cells express an abundant level of εBP protein, and therefore we explored a cDNA library derived from HeLa cell mRNA in order to obtain a full-length cDNA clone. Several clones were again identified, and partial sequencing revealed they were siblings containing inserts of nearly equal length. One of the clones (2.2) was completely sequenced and is reported in Figure 1. The cloned cDNA contains 18 bp of 5′-untranslated sequence which was unique compared to the 5′-noncoding sequence of clone 27.1, 750 bp of coding region, and 146 bp of 3′-untranslated sequence. A typical polyadenylylation signal (AATAAA) is found 14 bp from the 3′-terminus of the cDNA.

In Vitro Transcription and Translation of the Cloned cDNA. In order to demonstrate that cDNA clone 2.2 codes for a protein with the expected properties of εBP (i.e., lactose-binding activity), mRNA was transcribed from the cloned cDNA and then translated in vitro. The translation products

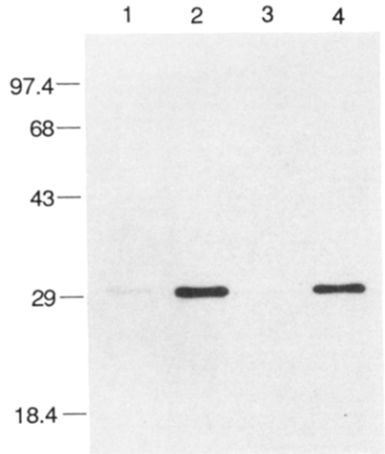

FIGURE 2: In vitro transcription and translation of cDNA clone 2.2 followed by fractionation on lactosyl-Sepharose. Clone 2.2 was transcribed and translated in a cell-free system using [^{35}S]methionine as label. Thereafter, translation products were incubated with either BSA–Sepharose followed by elution with 2% SDS (lane 1) or with lactosyl-Sepharose followed by elution with 2% SDS (lane 2), 0.2 M glucose (lane 3), or 0.2 M lactose (lane 4). Eluted proteins were analyzed by SDS–PAGE (10% polyacrylamide) and fluorography. The numbers on the left represent molecular weight ($\times 10^{-3}$) markers.

were absorbed with lactosyl-Sepharose, previously shown to effectively bind rat ϵBP, and subsequently eluted with buffers containing either SDS or lactose. As shown in Figure 2, a protein of M_r 30000 was detected by SDS–PAGE using either SDS (lane 2) or lactose (lane 4) elution but not in glucose-eluted (lane 3) samples. In addition, the protein did not bind significantly to BSA–Sepharose 4B (lane 1), indicating that, by comparison, the binding to lactosyl-Sepharose is specific. These results confirm that the cloned sequence indeed codes for a protein with lactose-binding activity.

High Conservation of ϵBP Protein Sequence. The amino acid sequence deduced from cDNA clone 2.2 is included in Figure 1. The sequence of human ϵBP, encoding a protein with a predicted molecular weight of 26.2K, does not contain a recognizable signal sequence nor a sequence characteristic of a transmembrane domain, and, as such, is similar to mouse and rat ϵBP. However, unlike the mouse and rat sequence, the predicted human sequence contains a potential N-linked glycosylation site (*Asn*XaaSer/Thr) at Asn^3. Whether this site is utilized or not has not been determined, but by SDS–PAGE and gel to gel comparison of in vitro translated ϵBP (nonglycosylated) to ϵBP isolated from either Wil-2 or HeLa cells, it appears that the apparent molecular weights are very similar, suggesting the potential N-linked glycosylation site is not utilized.

The human ϵBP sequence was compared to the corresponding rat (Albrandt et al., 1987) and murine (Jia & Wang, 1988) sequences using the IALIGN program, as shown in Figure 3. Of the 250 amino acids, 209 residues (84%) are identical with rat ϵBP, 205 residues (82%) are identical with murine ϵBP (CBP 35), and 220 residues (88%) are identical with either rat or murine ϵBP. The most noticeable difference found among the sequences is the absence of a 11-residue stretch in the human sequence within the amino-terminal portion. Significantly, this "deletion" was found in separate clones isolated from different libraries (normal human lung and HeLa cell), suggesting that it is neither a cloning artifact nor an isolated mutational event in the cells from which the original mRNA was derived.

Similar to rat and murine ϵBP, the human ϵBP sequence can be divided into two domains. The amino-terminal domain

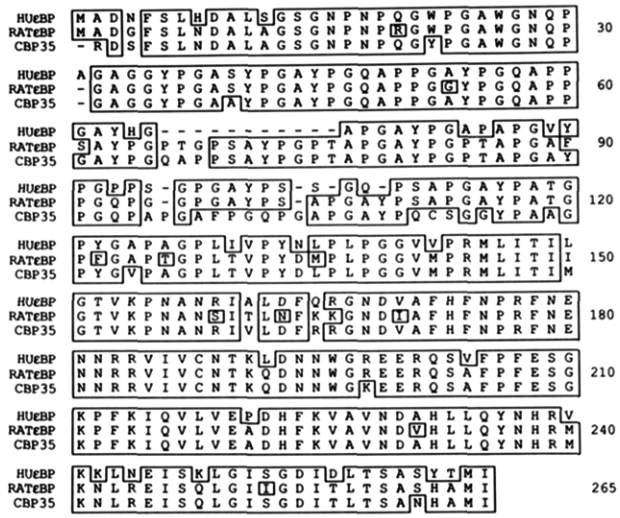

FIGURE 3: Comparison of amino acid sequences between human, mouse (CBP 35), and rat ϵBP. The sequences were positioned for best fit using the IALIGN program, and the single-letter amino acid code is used. Boxes indicate sequence identity, and numbers on the right margin indicate amino acid position.

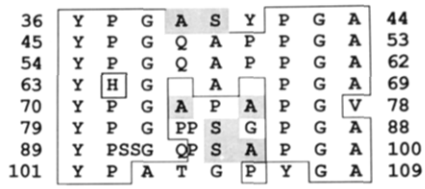

FIGURE 4: Human ϵBP contains a highly conserved repeated sequence in the amino-terminal domain. A nine amino acid segment is repeated 8 times.

is composed of highly conserved repeated sequences as shown in Figure 4. Due to the "deletion" of 11 residues mentioned above and 2 other gaps in the human sequence, as compared to rat ϵBP, there are only eight repeats compared to the 10 repeats noted for rat ϵBP. The consensus sequence, Tyr-Pro-Gly-(Gln/Ala)-(Ala/Ser)-(Pro/Ala)-Pro-Gly-Ala, is also slightly different from that of rat ϵBP with the most notable difference found at the fifth amino acid position (consensus Ala/Ser in human ϵBP compared to Ala/Thr in rat ϵBP). The carboxyl-terminal domain, on the other hand, is highly conserved. Particularly striking is the region between Arg^{166} and Arg^{239} where only 6 differences are found in the 74-residue segment (92% identity) between human and rat ϵBP and only 4 differences (95% identity) between human ϵBP and CBP 35. Moreover, within this region, all residues are shared by at least two of the species compared. Finally, it should be pointed out that both rat and human ϵBP have a single cysteine residue while mouse ϵBP (CBP 35) has a second in addition to the conserved site.

Cell Type Specific Expression of Human ϵBP mRNA. A Northern blot analysis was performed using the cloned human ϵBP cDNA to probe RNA isolated from HeLa cells. As shown in Figure 5, lane 2, a predominant hybridizing band of 1.1 kb was detected which is similar in size to that previously reported for rat ϵBP mRNA (Liu et al., 1985) (see lane 1).

Previously, we demonstrated the expression of ϵBP in a variety of tissues and different cell lines. With the human ϵBP clone in hand, we did an initial survey of selected human cell lines for the presence of ϵBP mRNA. As shown in Figure 5, a spectrum of ϵBP mRNA levels was detected: a human B-cell line (Wil-2) showed the highest level (lane 5) followed by a very low level in the monocyte line, U937 (lane 3), and no detectable ϵBP mRNA in another B-cell line, RPMI 8866

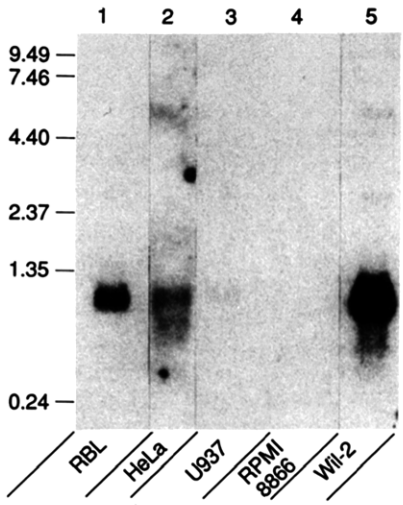

FIGURE 5: Northern blot of mRNA derived from various cell lines. Poly(A+) RNA was electrophoresed on a 1% agarose–formaldehyde gel, blotted, and probed with a human εBP cDNA fragment spanning the εBP coding region. The following mRNAs were probed: RBL (5 μg), lane 1; HeLa (2.5 μg), lane 2; U937 (5 μg), lane 3; RPMI 8866 (5 μg), lane 4; Wil-2 (5 μg), lane 5. Numbers on the left margin represent RNA molecular weight ($\times 10^{-3}$) markers.

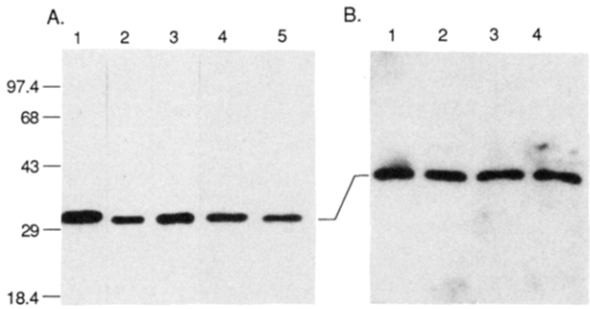

FIGURE 6: Immunoblot of human εBP isolated from HeLa cells or Wil-2 cells and affinity-purified using lactosyl-Sepharose or murine IgE–Sepharose. (Panel A) HeLa cell protein extracts were affinity-purified (i) using lactosyl-Sepharose 4B with bound protein subsequently eluted with SDS–PAGE sample buffer (lane 2) or 0.2 M lactose (lane 3) or (ii) using murine IgE–Sepharose 4B and elution with SDS (lane 4) or 0.2 M lactose (lane 5). Lane 1 is rat εBP derived from RBL cells, affinity-purified with lactosyl-Sepharose 4B, and eluted with SDS. (Panel B) Wil-2 protein extracts were affinity-purified using murine monoclonal anti-DNP IgE–Sepharose 4B (lane 1, SDS elution; lane 2, 0.2 M lactose elution) or lactosyl-Sepharose 4B (lane 3, SDS elution; lane 4, 0.2 M lactose elution). The isolated proteins were analyzed by SDS–PAGE (10% in panel A, 12.5% in panel B) followed by immunoblotting. Numbers on the left margin denote molecular weight ($\times 10^{-3}$) markers.

(lane 4). When clone 27.1 cDNA (which contains a partial ferritin gene sequence, as mentioned above) was used, an intense hybridizing band at ~1.0 kb (which we assume to be ferritin mRNA) was detected in the RNA blot of RPMI 8866 and U937 RNA, supporting the integrity of the RNA preparation (data not shown). Additional RNA blotting experiments revealed either very low or nondetectable levels of εBP in the DAUDI human B cell line and the HUT78 human T-cell line, respectively (data not shown).

Isolation of Human εBP Protein. We next performed experiments to show the existence of human εBP protein in cells. A monospecific antibody, previously generated against a peptide sequence in rat εBP (Gritzmacher et al., 1988) (now known to be identical in human εBP), was a particularly useful probe for immunoblotting studies. Protein extracts from HeLa cells were subjected to affinty purification with murine IgE–Sepharose 4B or lactosyl-Sepharose 4B, and eluted protein was analyzed by SDS–PAGE followed by immunoblotting using

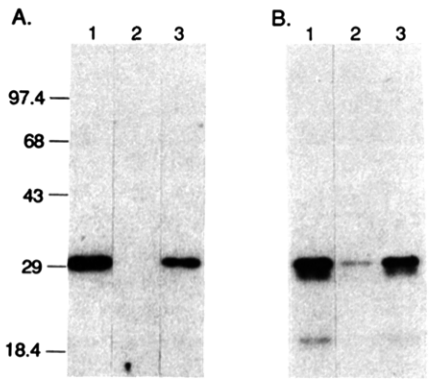

FIGURE 7: Recognition of εBP by human and murine IgE–Sepharose 4B. (Panel A) Immunoblot of εBP, derived from HeLa extracts, bound to either neuraminidase-treated murine IgE–Sepharose 4B (lane 1), human myeloma IgE(PS)–Sepharose 4B (lane 3), or untreated IgE(PS)–Sepharose 4B (lane 2) followed by 0.2 M lactose elution. (Panel B) Immunoreactivity of ^{125}I-labeled recombinant rat εBP with neuraminidase-treated murine IgE–Sepharose 4B (lane 1), human IgE(PS)–Sepharose 4B (lane 3), or untreated IgE(PS)–Sepharose 4B (lane 2) followed by 0.2 M lactose elution. Both panels show protein resolved by 10% SDS–PAGE followed by either immunoblotting (panel A) or autoradiography (panel B). Numbers on the left margin denote molecular weight ($\times 10^{-3}$) markers.

the sequence-specific antibody. As shown in Figure 6 (panel A), a protein band of M_r 30 000 was detected from both adsorbents eluted with SDS or lactose (lanes 2–5). By comparison with rat εBP (lane 1), human εBP appears to migrate slightly faster, perhaps due to the 11-residue "deletion" found for human εBP. The results also suggest either that the potential glycosylation site predicted in human εBP is not utilized or that the glycosylation does not significantly increase the apparent molecular weight of human εBP. The combination of using specific ligand (IgE–Sepharose and lactosyl-Sepharose) and sequence-specific antibodies, together with the molecular size, is highly supportive that the detected protein is human εBP.

Protein extracts from Wil-2 cells were similarly analyzed, using murine IgE–Sepharose in addition to lactosyl-Sepharose. As shown in Figure 6 (panel B), εBP bound essentially equivalently to either murine IgE–Sepharose 4B (lanes 1 and 2) or lactosyl-Sepharose 4B (lanes 3 and 4) and was eluted by either SDS (lanes 1 and 3) or lactose (lanes 2 and 4) from both affinity supports. These results further establish the carbohydrate-binding activity of this protein and also cross-species reactivity of human εBP with murine IgE.

Recognition of Different Human IgE Glycoforms by Human εBP. Having established reactivity of human εBP to both lactosyl-Sepharose 4B and murine IgE–Sepharose 4B, we then investigated reactivity to myeloma human IgE(PS)–Sepharose 4B. Surprisingly, human εBP derived from HeLa extracts did not bind to the immobilized myeloma IgE(PS) as shown by immunoblot analysis (Figure 7, panel A, lane 2). A similar lack of reactivity was found for two other human myeloma IgEs (DZA and BEDORA) with HeLa-derived εBP in addition to no discernible recognition of εBP, produced from Wil-2 cells, by IgE(PS)–Sepharose (data not shown).

From a carbohydrate mapping study, it is known that myeloma IgE(PS) is extensively sialyated (Baenziger et al., 1974). Because sialic acid (*N*-acetylneuraminic acid) residues are typically found attached to terminal galactose residues in glycoproteins, we considered the possibility that lack of reactivity of myeloma IgE(PS) to εBP was due to the presence of sialic acid residues blocking presentation of the appropriate galactose-capped oligosaccharide determinants. Thus, myeloma IgE(PS)–Sepharose was treated with neuraminidase and

then evaluated for reactivity to εBP. As shown in Figure 7, panel A, the use of neuraminidase-treated human IgE(PS)–Sepharose (lane 3) resulted in a significant degree of recognition between human IgE and εBP. An identical pattern of reactivity was also found for two other human myeloma IgEs, DZA and BEDORA (data not shown). In a parallel experiment (Figure 7, panel B), ^{125}I-labeled recombinant rat εBP was shown to exhibit very slight reactivity to IgE(PS)–Sepharose (lane 2) but pronounced binding to neuraminidase-treated IgE(PS)–Sepharose (lane 3). The results indicate that desialylated human myeloma IgE exhibits recognition of εBP and that this recognition is largely blocked by sialylation of IgE carbohydrate chains.

Discussion

This paper reports the isolation from human HeLa cells of cDNA coding for human IgE-binding protein. The authenticity of the cloned cDNA is supported by the following observations: (i) mRNA transcribed from the cDNA can be translated in vitro to afford a protein similar in size to rat εBP and with lactose-binding activity; (ii) the deduced amino acid sequence showed extensive sequence homology to rat and murine εBP; (iii) like rat and murine εBP, the deduced protein sequence of human εBP is also composed of a two-domain structure with the amino-terminal domain containing tandem repeats of a highly conserved amino acid sequence; (iv) the carboxyl-terminal domain exhibited significant homology with consensus sequences from a family of S-type lectins; (v) the cloned cDNA hybridizes with mRNA from several human cell lines which was similar in size to rat εBP mRNA; (vi) a protein of M_r 30 000 was isolated from two human cell lines which is recognized by antibodies directed to a peptide sequence predicted in human εBP. In addition, we demonstrate that human εBP, like rat and murine εBP, binds to murine IgE–Sepharose and lactosyl-Sepharose and the binding can be reversed by lactose. We thus conclude that human εBP is also an endogenous lectin with β-galactoside-binding activity.

Several observations accrued during this study to support the contention that cDNA clone 2.2 contains the entire coding sequence for the mature protein: (i) the position of the translation initiation methionine (and adjacent coding sequence) for the rat (Albrandt et al., 1987) and murine (Raz et al., 1989; Cherayil et al., 1989) homologues aligns quite favorably with the start methionine proposed for clone 2.2; (ii) the apparent molecular weight of in vitro translated εBP, as determined by SDS–PAGE and gel to gel comparison, is very similar to that found for εBP derived from several human cell lines. As an extension of these observations, it is reasonable to infer that the cDNA sequence previously reported for rat εBP (Albrandt et al., 1987) also contains full-length coding sequence despite the small discrepancy reported for the apparent molecular weight of in vitro translated rat εBP compared to εBP isolated from RBL cells (Albrandt et al., 1987).

An interesting finding in this study is the significant conservation of the amino acid sequence among the three animal species compared. This pertains to both the amino-terminal and carboxyl-terminal domains, suggesting both parts are important for the function of this protein. The amino-terminal domain exhibits sequence similarity to protein components of hnRNP; in fact, it has been demonstrated that CBP 35 (equivalent to εBP) forms complexes with nuclear RNA (Laing & Wang, 1988). By FASTP analysis, a generally similar degree of homology (20% identity) was found between human εBP and the hnRNP sequences surveyed by Wang et al. (Jia & Wang, 1988). Whether this homology reflects an evolutionary relationship between these proteins is not known.

Comparison of the sequence in the carboxyl-terminal domain of εBP among the three species is quite informative. The region between Arg^{166} and Arg^{239} is essentially completely conserved as detailed under Results. This area has been previously noted (Liu, 1990; Jia & Wang, 1988) to possess significant homology between εBP/CBP 35 and another group of S-type soluble lectins of M_r 14K–16K (Clerch et al., 1988; Raz et al., 1988; Southan et al., 1987; Gitt & Barondes, 1986; Paroutaud et al., 1987). In particular, one stretch of amino acids (HFNPRF, amino acids 173–178, human εBP) is invariant for εBP derived from the three species and identical with a consensus sequence of the 14–16-kDa lectins (Levi & Teichberg, 1981). An additional human εBP sequence (*W*GX*E*ERQ; amino acids 196–202) is also highly conserved compared to the rat and murine sequences and exhibits conserved Trp and Glu residues (italicized) previously implicated as critical sites for lectin activity (Levi & Teichberg, 1981). Together these comparisons indicate a high level of conservation in regions of putative carbohydrate-binding activity which may, in turn, indicate some relationship between these lectins in specificity and/or function.

Another interesting finding is the cell-specific expression of εBP in various lymphoid cell lines. It appears that expression of εBP may be dependent on lymphocyte differentiation, as the three cell lines tested express varying cell-surface markers that represent different stages of B-cell differentiation (for Wil-2, FcεR^+, FcγR^+, Ig nonproducer; for RPMI-8866, FcεR^+, FcγR^-, IgG producer; for DAUDI, FcεR^-, FcγR^+, Ig nonproducer). The expression of CBP 35 has been shown to be developmentally regulated in that the protein is abundant in certain embryonic tissues but low in corresponding tissues in the adult (Crittenden et al., 1984). In addition, the role of lectins and glycoconjugates in cell differentiation has been well documented (Feizi, 1985; Sharon & Lis, 1989). Therefore, differentiation-dependent expression of εBP is certainly possible. Significantly, expression of the Mac-2 antigen, which is identical with CBP 35 (Cherayil et al., 1989), has been shown to increase during maturation of macrophage precursors (Leenen et al., 1986) which further supports a link between this protein and cell development. Further investigation will be necessary to establish a correlation between εBP expression and lymphocyte differentiation.

The initial characterization of protein corresonding to human εBP resulted in the demonstration of both carbohydrate-binding activity and cross-species reactivity to murine IgE. Both results parallel the reactivity pattern shown by the murine (CBP 35) and rat homologues and are consistent with the highly conserved interspecies amino acid sequence, particularly within the carboxy terminus, of these proteins. Perhaps the most interesting finding in this study, however, was the pattern of reactivity found between εBP and human IgE. The lack of binding of human εBP to three different human myeloma IgE proteins was, initially, quite surprising. However, it did provide a clue to the existence of carbohydrate heterogeneity among IgE proteins. Since human εBP does bind murine IgE as well as neuraminidase-treated human myeloma IgE, we concluded that the lack of significant reactivity of human εBP to untreated myeloma IgE is most likely due to the masking of the εBP ligand in IgE, i.e., galactosides, by sialylation. In fact, it has been shown by Sparrow et al. (1987) that HL-29 (which is likely the equivalent of human εBP) binds lactose to a much greater extent than lactose derivatized with sialic acid (6-sialolactose).

One possible explanation for the observed εBP selectivity in IgE recognition is that oligosaccharide chains present on

human IgE are significantly different compared to murine IgE and this difference precludes recognition of human IgE by εBP. This phenomenon may indicate that there is a species-dependent pattern of reactivity between IgE and εBP. Alternatively, the myeloma IgEs tested may not be representative of normal polyclonal IgE, particularly with regard to structure and composition of carbohydrates. Indeed, it has been documented that proteins from transformed cells, including in vivo tumors, often exhibit different carbohydrate composition and structure relative to nontransformed cells (Hakomori, 1984). Moreover, this difference has been shown to take the form of excessive sialylation of O-linked oligosaccharides in some examples (Hakomori, 1984). In either event, it should be informative to evaluate εBP reactivity to samples of polyclonal IgE derived from nontransformed cells.

The significance of IgE-binding activity for εBP remains an interesting question. Since demonstration of the lectin property for εBP, it became clear that IgE binding may be accounted for by the recognition of carbohydrates on IgE. In addition, this recognition has been reported to show isotype specificity (Cherayil et al., 1989) which is in agreement with our own findings (unpublished results). Therefore, with the demonstrated recognition of murine IgE and neuraminidase-treated human IgE by εBP, a possible function in the IgE system cannot be excluded. It is interesting to contrast εBP to the low affinity IgE receptor (FcεRII) which also contains a lectin domain (Kikutani et al., 1986; Ikuta et al., 1987; Ludin et al., 1987). One significant difference between the two is that FcεRII appears to recognize IgE protein sequence and its binding to IgE apparently is not carbohydrate dependent (Vercelli et al., 1989). However, FcεRII has been shown to bind carbohydrates (Richards & Katz, 1990), and its functions may not be limited to those mediated through recognition of IgE. A particularly attractive possibility for εBP function in the IgE system may be a role in augmenting IgE-dependent mast cell activation. Recent demonstration that the Mac-2 antigen (equivalent to CBP35/εBP) is found, in part, to be secreted into the extracellular space (Cherayil et al., 1989) suggests that, under the appropriate stimulus, εBP may respond similarly. In the event the εBP could be secreted, it is conceivable that, through IgE binding, it might activate IgE-bound mast cells, resulting in degranulation in an antigen-independent manner. These possibilities await future investigation.

The physiological role(s) of εBP (CBP 35, L-34, RL-29, and Mac-2) is (are) intriguing. So far, studies by other investigators have related this lectin to growth regulation, cell transformation, metastasis, and, in macrophages, inflammatory signals. The detection of this lectin in the cytoplasm, nucleus, and cell surface suggests it may have multiple functions. Isolation of human εBP cDNA reported herein established the existence of the human counterpart of εBP and revealed a high conservation of protein sequence. Detection of a spectrum of εBP mRNA levels from several B-lymphocyte cell lines was found and may reflect a link between lymphocyte differentiation and εBP expression. The cDNA described herein should facilitate the analysis of both gene expression of this protein in various physiological and pathological conditions in human systems and structure–function correlates of εBP, thereby improving our understanding of the function(s) of this lectin.

Acknowledgments

We thank Dr. R. Ogata for critically reviewing the manuscript, Dr. K. Ishizaka and Dr. H. Spiegleberg for generous gifts of myeloma IgE, and Janet Czarnecki for excellent assistance in the preparation of the manuscript.

References

Agrwal, W., Wang, J. L., & Voss, P. G. (1989) *J. Biol. Chem. 264*, 7236.

Albrandt, K., Orida, N. K., & Liu, F.-T. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6859.

Baenziger, J., Kornfeld, S., & Kochwa, S. (1974) *J. Biol. Chem. 249*, 1897.

Barondes, S. H. (1988) *Trends Biochem. Sci. 13*, 480.

Cherayil, B. J., Weiner, S. J., & Pillai, S. (1989) *J. Exp. Med. 170*, 1959.

Clerch, L. B., Whitney, P., Hass, M., Brew, K., Miller, T., Werner, R., & Massaro, D. (1988) *Biochemistry 27*, 692.

Crittenden, S. L., Roff, C. F., & Wang, J. L. (1984) *Mol. Cell. Biol. 4*, 1252.

Drickamer, K. (1988) *J. Biol. Chem. 263*, 9557.

Feizi, T. (1985) *Nature 314*, 53.

Gitt, M. A., & Barondes, S. H. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 7603.

Gritzmacher, C. A., Robertson, M. W., & Liu, F.-T. (1988) *J. Immunol. 141*, 2801.

Hakomori, S. (1984) *Annu. Rev. Immunol. 2*, 103.

Hanahan, D., & Meselson, M. (1980) *Gene 10*, 63–67.

Ikuta, K., Takami, M., Kim, C. W., Honjo, T., Miyoshi, T., Tagaya, Y., Kawabe, T., & Yodoi, J. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 819.

Jia, S. & Wang, J. L. (1988) *J. Biol. Chem. 263*, 6009.

Kikutani, H., Inui, S., Sato, R., Barsumian, E. L., Owaki, H., Yamasaki, K., Kaisho, T., Uchibayashi, N., Hardy, R. R., Hirano, T., Tsunasawa, S., Sakiyama, F., Suemura, M., & Kishimoto, T. (1986) *Cell 47*, 657.

Laemmli, U. K. (1970) *Nature 227*, 680.

Laing, J. G., & Wang, J. L. (1988) *Biochemistry 27*, 5329.

Laing, J. G., Robertson, M. W., Gritzmacher, C. A., Wang, J. L., & Liu, F.-T. (1989) *J. Biol. Chem. 264*, 1907.

Leenen, P. J. M., Jansen, A. M. A. C., & van Ewijk, W. (1986) *Differentiation 32*, 157.

Leffler, H., & Barondes, S. H. (1986) *J. Biol. Chem. 261*, 10119.

Leffler, H., Masiarz, F. R., & Barondes, S. H. (1989) *Biochemistry 28*, 9222.

Levi, G., & Teichberg, V. I. (1981) *J. Biol. Chem. 256*, 5735–5740.

Liu, F.-T. (1990) *CRC Crit. Rev. Immunol. 10*, 289.

Liu, F.-T., & Orida, N. (1984) *J. Biol. Chem. 259*, 10649.

Liu, F.-T., Bohn, J. W., Ferry, E. L., Yamamoto, H., Molinaro, C. A., Sherman, L., Klinman, N. R., & Katz, D. H. (1980) *J. Immunol. 124*, 2728–2737.

Liu, F.-T., Albrandt, K., Mendel, E., Kulczycki, A., Jr., & Orida, N. K. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4100.

Liu, F.-T., Albrandt, K., & Robertson, M. W. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 5639.

Lüdin, C., Hofstetter, H., Sarfati, M., Levy, C. A., Suter, U., Alaimo, D., Kilchherr, E., Frost, H., & Delespesse, G. (1987) *EMBO J. 6*, 109.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

McConahey, P. J., & Dixon, F. J. (1966) *Int. Arch. Allergy 29*, 185.

Moutsatsos, I. K., Wade, M., Schindler, M., & Wang, J. L. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6452.

Paroutaud, P., Levi, G., Teichberg, V. I., & Strosberg, A. D. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6345.

Raz, A., & Lotan, R. (1987) *Cancer Metastasis Rev. 6*, 433.

Raz, A., Carmi, P., & Pazerini, G. (1988) *Cancer Res. 48*, 645.
Raz, A., Pazerini, G., & Carmi, P. (1989) *Cancer Res. 49*, 3489.
Richards, M. L., & Katz, D. H. (1990) *J. Immunol. 144*, 2638.
Roff, C. F., & Wang, J. L. (1983) *J. Biol. Chem. 258*, 10657.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463.
Sharon, N., & Lis, H. (1989) *Science 246*, 227.
Southan, C., Aitken, A., Childs, R. A., Abbott, W. M., & Feizi, T. (1987) *FEBS Lett. 214*, 301.
Sparrow, C. P., Leffler, H., & Barondes, S. H. (1987) *J. Biol. Chem. 262*, 7383.
Vercelli, D., Helm, B., Marsh, P., Padlan, E., Geha, R. S., & Gould, H. (1989) *Nature 338*, 649.

Protection from Photoinhibition by Low Temperature in *Synechocystis* 6714 and in *Chlamydomonas reinhardtii*: Detection of an Intermediary State

Diana L. Kirilovsky,* Claudie Vernotte, and Anne-Lise Etienne
UPR 407, Bâtiment 24, CNRS, 91198 Gif sur Yvette, France
Received March 21, 1990; Revised Manuscript Received May 17, 1990

ABSTRACT: Photoinhibition was induced in a cyanobacterium strain, *Synechocystis* 6714, and a green alga, *Chlamydomonas reinhardtii*, by exposing them to light intensities from 1000 to 4000 $\mu E/(m^2 \cdot s)$ at various temperatures. The photoinhibition process was followed by measurements of chlorophyll fluorescence and oxygen evolution. During exposure to high light, fluorescent active reaction centers II became low fluorescent inactive centers. This process involved several reversible and irreversible steps. The pathway from the active state to the inactive low fluorescent state was different in *Synechocystis* and *Chlamydomonas*. In the latter there was a reversible intermediary step characterized by an increase of F_0. This state was stable at 5 °C and slowly reversible at room temperature. The high F_0 fluorescence level corresponded to a state of photosystem II centers that were inactive for oxygen evolution. An F_0 decrease occurred in the dark in the absence of protein synthesis and was correlated to a restoration of oxygen evolution. Further experiments suggested that the existence of the intermediate fluorescent state is due to modified closed centers in which the reduced primary acceptor is less accessible to reoxidation. In cyanobacteria this reversible state was not detected. In both organisms, the decrease of F_{max} reflected an irreversible damage of photosystem II centers. These centers need replacement of proteins in order to be active again. The quenching of F_{max} and the irreversible inhibition of oxygen evolution were slowed down in both organisms by decreasing the temperature of the photoinhibitory treatment from 34 to 5 °C. We conclude that low temperature protected the reaction center II from irreversible photodamage.

Photoinhibition is related to the excess of light absorbed by the pigment antennae which cannot be properly dissipated by photosynthesis (Osmond, 1981; Powles, 1984; Kyle, 1987; Cleland, 1988). There is now a large amount of evidence demonstrating that the primary site of lesion is the reaction center of the photosystem II (PSII).[1] Different sites in the reaction center II (RCII) were proposed to be the first target of high light. Studies on isolated chloroplasts, thylakoids, or PSII preparations suggested that the P_{680}–Phe–Q_A portion of the electron transport is the primary site of damage (Cleland et al., 1986; Theg et al., 1986; Arntz & Trebst, 1986; Vass et al., 1988; Styring et al., 1990). As opposed to that, results obtained with intact organisms suggested that the Q_B niche in the D_1 protein is the first site to be damaged (Kyle et al., 1984; Kirilovsky et al., 1988; Ohad et al., 1988). It was observed that the electron transfer through Q_B ($H_2O \rightarrow$ DCBQ or $H_2O \rightarrow$ DCIP) decreased more rapidly than electron transfer which did not involve Q_B ($H_2O \rightarrow$ SiMo) (Kirilovsky et al., 1988, Kyle et al., 1984). Moreover, thermoluminescence measurements showed that modifications of the B band appeared before modifications of the Q band. B and Q signals result from the charge recombination between $S_{2,3}$ and Q_B^- and Q_A^-, respectively (Ohad et al., 1988; Kirilovsky, Ducruet, and Etienne, unpublished data).

The decrease of PSII activity due to photoinhibition can be restored if the cell exposure to high light is not too long. The repair process, which is light dependent, involves de novo synthesis of thylakoid proteins among which the most prominent is D_1 (Ohad et al., 1985; Lönneborg et al., 1988; Kirilovsky et al., 1988).

It is assumed that environmental conditions that reduced the rate of photosynthesis accentuate the effects produced by plants exposed to high light. Exposure of leaves of many plants to high photon flux densities at chilling temperatures produces a damage to the photosynthetic apparatus which is greater than that observed at higher temperatures [reviewed by Oquist et al. (1987)]. The recovery is also temperature dependent, being slower at low temperatures. It was proposed that the inhibition of recovery by low temperatures may also contribute to the particular susceptibility to photoinhibition in plants at chilling

[1] Abbreviations: Chl, chlorophyll; D_1 and D_2, polypeptides of the RCII; DCBQ, dichlorobenzoquinone; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_0, F_v, F_{max}, initial, variable, and maximum fluorescence; P_{680}, a chlorophyll molecule that acts as the primary electron donor in the RCII; Pheo, pheophytin, intermediary electron acceptor; PSII, photosystem II; Q_A and Q_B, primary and secondary quinone electron acceptors, respectively; RCII, reaction center II; Z, electron donor to P_{680}.

0006-2960/90/0429-8100$02.50/0 © 1990 American Chemical Society