We analyzed mouse hepatoma cells using differential display to discover new genes that respond to the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We identified a class I major histocompatibility complex (MHC) gene, which we designated as MHC Q1b, whose expression decreases in the presence of TCDD. TCDD-induced down-regulation of MHC Q1b requires both the aromatic hydrocarbon receptor and the aromatic hydrocarbon receptor nuclear translocator, transcription factors that up-regulate other genes in response to TCDD. Down-regulation of MHC Q1b by TCDD appears to involve both transcriptional and post-transcriptional regulatory events; the post-transcriptional destabilization of MHC Q1b mRNA is probably a secondary response to TCDD. Our findings reveal new mechanistic aspects of gene regulation by TCDD. In addition, our observations suggest a mechanism that might account for some of TCDD's immunotoxic effects.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) represents the prototype for a class of structurally related halogenated aromatic hydrocarbons, including polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls. Many such compounds are widespread environmental contaminants, produce similar patterns of toxicity, and appear to share the same receptor-mediated mechanism of action. Because TCDD is the most potent, it has been studied much more extensively than other compounds. TCDD is generated during combustion processes, enters the atmosphere, contaminates the food chain, and persists in the environment, because it is resistant to biological degradation. In animals, TCDD elicits numerous adaptive and/or adverse effects, including enzyme induction, epithelial hyperplasia, teratogenesis, tumor promotion, liver toxicity, thymic atrophy, and immunosuppression. In humans, the most well documented adverse response to TCDD is chloracne; there is also concern that TCDD produces cancer, birth defects, and immunosuppression. In marsupials, the most well documented adverse response to TCDD is chloracne; there is also concern that TCDD produces cancer, birth defects, and immunosuppression (1–3). The molecular mechanism of TCDD action probably involves alterations in gene expression, which are mediated by the aromatic hydrocarbon receptor (AhR). AhR is a cytoplasmic, basic helix-loop-helix protein that binds TCDD saturably and with high affinity, thereby activating signaling pathways that modulate gene expression (4, 5).

The best understood AhR-regulated response to TCDD is the induction of CYP1A1 gene transcription (5). CYP1A1 encodes the microsomal enzyme cytochrome P4501A1, which catalyzes the oxygenation of lipophilic aromatic hydrocarbons during their metabolic processing to water-soluble derivatives (6). TCDD binds to AhR in the cytoplasm; subsequently, the ligand-bound AhR enters the nucleus and heterodimerizes with a second basic helix-loop-helix protein known as the AhR nuclear translocator (Arnt). The AhR/Arnt heterodimer functions as a ligand-dependent transcription factor; it binds to a specific DNA sequence, termed a xenobiotic-responsive element, within an enhancer upstream of the CYP1A1 gene (7, 8). This protein-DNA interaction is associated with disruption of nucleosomes, occupancy of the CYP1A1 promoter by transcription factors, and activation of gene expression (9–11). In comparison with activation of transcription, we know relatively little about the down-regulation of gene expression by TCDD.

One way to increase our understanding of TCDD action is to analyze additional TCDD-responsive genes. To address this issue experimentally, we used differential display, a versatile method that can identify both up- and down-regulated genes (12, 13). We studied mouse hepatoma cells, because the availability of AhR-defective and Arnt-defective mutants permits both genetic and biochemical analyses of the regulatory mechanism (14, 15). Here, we show that TCDD down-regulates the expression of a class I major histocompatibility complex (MHC) gene, which we designate as MHC Q1b, and we examine the mechanism of the down-regulation.

EXPERIMENTAL PROCEDURES

Materials—RNAimage kits for differential display were from GenHunter (Nashville, TN). Tag polymerase was from Perkin-Elmer. Restriction endonucleases, T4 DNA ligase, and polynucleotide kinase were from Life Technologies, Inc., Promega (Madison, WI), and New England Biolabs (Beverly, MA). [γ-32P]ATP, [α-32P]UTP, [α-32P]dATP, and [35S]dATP were from Amersham Corp. Total RNA isolation kits and DNA purification kits were from Qiagen (Chatsworth, CA). The in vitro transcription kit was from Ambion (Austin, TX). The DNA sequencing kit (Sequenase version 2.0) was from U.S. Biochemical Corp. Digoxigenin (DIG)-UTP, DIG-DNA, and DIG-RNA labeling and detection kits and CDPhostar substrate were from Boehringer Mannheim. A TA cloning kit was from Invitrogen (San Diego, CA).

Cell Culture—Wild-type (Hepa 1c1c7), AhR-defective, and Arnt-defective cells were maintained in minimal essential medium containing 10% fetal bovine serum as described (14, 16).

Differential Display—We used a reverse transcription-PCR-based differential display approach (12, 13). Experiments were carried out using the RNAimage kit according to the manufacturer’s instructions (GenHunter). Briefly, total RNA was isolated from untreated or TCDD-treated (1 nM, 16 h) wild-type cells using a Qiagen total RNA isolation kit and was reverse-transcribed using as primers one of the three different one base-anchored oligo(dT)16 primers, H-TM (where M may be A, C, or G), containing a HindIII site. cDNAs were amplified by PCR in the presence of [32P]dATP using the H-TM primer and a 13-mer arbitrary primer, H-AP, containing a HindIII site for subcloning. PCR

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products were separated on a 6% DNA sequencing gel and detected by autoradiography. Bands exhibiting differential expression were excised from the dried gel, and DNAs on the gel slice were eluted and amplified by PCR using the same set of primers. The PCR products were subcloned into a TA cloning vector, pCR II or pCR 2.1 (Invitrogen), and sequenced using a Sequenase kit. Tag polymerase was used in all PCR steps. The conditions for reverse transcription and PCR were as recommended by the manufacturer (GenHunter).

cDNA Library Screening—To obtain full-length cDNA, we screened a Hepa 1c1c7 cell cDNA library (17), using clone C38 from the differential display (see “Results”). A DIG-labeled DNA probe was synthesized according to the manufacturer’s instructions (Boehringer Mannheim), using clone 38 as a template. The labeled probe was used to screen 1 × 10^6 phage plaques. Positive clones, contained within the pBK-CMV phagemid, were excised in vivo from the ZAP Express vector using the ExAssist-SOLR system, as recommended by the manufacturer (Stratagene). The excised phagemids containing the positive clones were sequenced by the dyeideoxy method using a Sequenase kit.

RNA Analyses—For Northern blotting, a cDNA fragment encoding exons 5 and 6 of MHC Q1b was generated by PCR, subcloned into pBluescript, and used as a template for riboprobe synthesis. Riboprobes were synthesized in the presence of DIG-UTP using a DIG RNA-labeling kit (Boehringer Mannheim). Total RNA was isolated from cells using a Qiagen total RNA isolation kit. RNA (10 μg) was electrophoresed on a 1% agarose-formaldehyde gel and transferred to nitrocellulose membrane (Schleicher & Schuell). After cross-linking, the membrane was hybridized with DIG-labeled riboprobe at 68 °C for 16 h; signals were detected by chemiluminescence using a DIG RNA labeling and detection kit with CDP Star as substrate (Boehringer Mannheim).

RNase Protection—cDNAs in pCR II or pCR 2.1 were used as DNA templates to synthesize 32P-labeled riboprobes. A cDNA fragment encoding exon 2 of MHC H-2Kb or Q1b, or Q10b was generated by PCR, subcloned into pBluescript, sequenced, and used for riboprobe synthesis. The DNA templates for MHC H-2Kb (18), H-2Db (19), and Q10b (20) were gifts from Dr. Larry R. Pease (Mayo Clinic). The DNA template for MHC Q1b was obtained from cDNA library screening. The transcription reaction was carried out in vitro in the presence of [α-32P]UTP using an Ambion transcription kit. Total RNA (5 μg) was hybridized with a riboprobe at 50 °C for 16 h. RNA was used as a negative control. Single-stranded RNA was digested with RNase A and RNase T1. Protected fragments were separated on a 6% polyacrylamide/urea gel and detected by autoradiography. A 1-kb DNA ladder was phosphorylated and electrophoresed in a parallel lane to estimate fragment size.

Nuclear Run-on—Nuclear run-on experiments were performed as described by Ausubel et al. (21) with modifications. Cells were lysed in 10 mM Tris-Cl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, and 0.5% Nonidet P-40. Nuclei were isolated by centrifugation for 5 min at 500 × g. In vitro transcription was carried out at 30 °C for 30 min in a transcription buffer containing 5 mM Tris-Cl, pH 8.0, 2.5 mM MgCl₂, 0.1 mM KCl, 0.1 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 0.175 mM UTP, and 0.325 mM DIG-UTP (Boehringer Mannheim). DIG-labeled RNA was purified using a Qiagen total RNA isolation kit. cDNAs immobilized on a nitrocellulose membrane were hybridized with DIG-labeled, purified RNA at 42 °C for 16 h. The membrane was washed, and the signal was detected by chemiluminescence according to the manufacturer’s recommendation (Boehringer Mannheim).

RESULTS

Identification of TCDD-regulated Genes by Differential Display—We employed a differential display technique to identify TCDD-responsive genes in Hepa 1c1c7 cells. As a positive control, we identified CYP1A1 (clone C17, Fig. 1), a gene that is known to be up-regulated by TCDD. In addition, we identified additional up-regulated genes and a down-regulated gene. We confirmed that TCDD regulates these genes using slot blot analyses and RT-PCR. Northern analysis data were not shown. These genes were cloned and sequenced. A GenBank search revealed that none of these cDNA sequences had been reported previously. We chose to analyze the down-regulated gene (clone C38, Fig. 1) because of the likelihood that such studies would reveal new mechanistic aspects of TCDD action.

Identification of a Full-length cDNA for Clone C38—To obtain full-length cDNA, we used clone C38 as a probe to screen a mouse hepatoma (Hepa 1c1c7) cDNA library (17). cDNAs that contained the C38 sequence were sequenced; a GenBank search indicated that the cDNAs are nearly identical to the MHC Q1b gene (mouse C3H strain) (22), a nonclassical, class I MHC gene (see “Discussion”), in all eight predicted exons (Fig. 2). At the 3’-end, all clones contain a poly(A) tail and a 528-nucleotide untranslated sequence; this untranslated region contains the clone C38 sequence, but it is not present in the MHC Q1b sequence. These observations explain why we did not find a sequence corresponding to C38 in GenBank. We have designated the down-regulated gene as MHC Q1b (mouse C57BL/6 strain).

Typically, mouse class I MHC genes contain eight exons, each of which encodes a separate protein domain (23). Exon 1 encodes a leader sequence. Exons 2 and 3 encode the extracellular α1 and α2 domains, which are involved in peptide binding. Exon 4 encodes the α3 domain, which interacts with β₂-microglobulin. Exon 5 encodes the transmembrane domain. Exons 6–8 encode the cytoplasmic domain. The sequences of seven cDNA clones for MHC Q1b imply that it exhibits substantial variation in RNA splicing, compared with MHC Q1b (22). For example, 1) one clone contains intron 1 (Fig. 3A), which could encode an open reading frame and could be in frame between exon 1 and exon 2; 2) four clones contain introns 5, 6, and/or 7 in various combinations (Fig. 3, B–D); 3) one clone lacks exon 3; 4) three clones are spliced from exon 5 to exon 8, resulting in deletion of exons 6 and 7. Only two of seven clones are normally spliced from exon 5 to exon 6. All clones contain exons 4 and 5, which encode the α3 domain and the transmembrane domain, respectively; these latter findings suggest that MHC Q1b may interact with β₂-microglobulin and may be membrane-anchored.

Comparison of DNA sequences in the predicted coding regions of Q1b and Q1k reveals four nucleotide mismatches, which produce one amino acid change; in addition, one nucleotide insertion in exon 7 of Q1k produces an open reading frameshift and the addition of five amino acids (Fig. 2). These sequence data suggest that MHC Q1 genes are relatively non-polymorphic, a characteristic that is typical of nonclassical class I MHC genes (20).

Expression of MHC Q1b mRNA—We performed Northern blots to determine the MHC Q1b transcript size. If the majority of transcripts contain eight exons plus the 528-nucleotide untranslated sequence, the transcript size should be ~2 kb. If the majority of transcripts contain unspliced introns identified in the cDNA clones, the transcript size should be ~2.7 kb. To identify an MHC Q1b-specific probe, we synthesized several probes by PCR using cDNA clones as templates, and we analyzed RNA from uninduced and TCDD-induced wild-type cells. Northern blots probed with exon 2, 3, or 4 showed two transcripts, about 1.6 and 2.7 kb in size. Expression of the 2.7-kb transcript was down-regulated by TCDD; in contrast, expres-
Down-regulation of MHC Q1b by TCDD

The 1.6-kb transcript was not affected by TCDD treatment (data not shown). Clone C38, exons 5 and 6, intron 5, and intron 6 hybridized only with the 2.7-kb transcript (data not shown). Because down-regulation of the 2.7-kb transcript by TCDD is not accompanied by the appearance of other transcripts or an increase in the 1.6-kb transcript, we infer that the TCDD-induced decrease in the 2.7-kb transcript is not due to a change in the pattern of RNA splicing. Our results indicate that Q1b mRNA is differentially spliced, that the 2.7-kb transcript represents most MHC Q1b mRNA, which contains unspliced introns, and that MHC Q1b mRNA is down-regulated by TCDD. The 1.6-kb transcript may represent cross-hybridization of exons 2–4 to H-2Db mRNA, which is 1.6 kb in size (24, 25) and is not regulated by TCDD, as described later. A probe containing exons 5 and 6 generated a strong signal with a low background; therefore, we used this probe for the remaining analyses.

Analyses of MHC Q1b mRNA from wild-type cells reveal that TCDD down-regulates MHC Q1b mRNA in a time-dependent manner (Fig. 4). Assuming a first order degradation rate, the half-life of MHC Q1b mRNA after TCDD treatment is about 7 h.

Fig. 2. Alignments of DNA sequences of the predicted exons and deduced amino acid sequences of the MHC Q1 genes from mouse C57BL/6 (Q1b) and mouse C3H (Q1k) strains. A dash indicates an identical nucleotide or amino acid, and an asterisk indicates a gap in the nucleotide sequence or a stop codon in the amino acid sequence. A nucleotide in parentheses indicates an insertion. AUREs are underlined. Predicted exons based upon the MHC Q1b DNA sequence from the mouse C3H strain (22) are as follows: exon 1, nucleotides 5–68; exon 2, nucleotides 69–338; exon 3, nucleotides 339–614; exon 4, nucleotides 615–890; exon 5, nucleotides 891–1025; exon 6, nucleotides 1026–1058; exon 7, nucleotides 1059–1106; exon 8, nucleotides 1107–1495; 3'-untranslated region, nucleotides 1496–2043.
In addition, the down-regulation of MHC Q1\textsuperscript{b} mRNA by TCDD is dose-dependent (Fig. 5). The EC\textsubscript{50} is about 30 pM, a value similar to that for the induction of CYP1A1 mRNA by TCDD, a response that is mediated by the Ah receptor.

AhR Dependence and Arnt Dependence of MHC Q1\textsuperscript{b} Down-regulation by TCDD—AhR and Arnt mediate most, if not all, responses to TCDD (5, 7, 8). Therefore, we asked whether down-regulation of MHC Q1\textsuperscript{b} expression by TCDD requires AhR and/or Arnt. We employed variant cells defective in either AhR or Arnt to address this question.

Our findings indicate that TCDD fails to down-regulate MHC Q1\textsuperscript{b} in Arnt-defective cells; down-regulation is restored by reconstitution of Arnt-defective cells with wild-type Arnt cDNA but not by reconstitution with a mutant Arnt cDNA (Fig. 6). These results indicate that down-regulation of MHC Q1\textsuperscript{b} by TCDD requires Arnt.

For reasons that we do not understand, AhR-defective cells do not express MHC Q1\textsuperscript{b} mRNA (data not shown). Therefore, we cannot use AhR-defective cells to study MHC Q1\textsuperscript{b} gene regulation. Instead, we used wild-type cells in which we expressed a mutant form of AhR, R39A, which has a dominant negative effect on TCDD-inducible gene expression (16). The mutant AhR can heterodimerize with Arnt to generate a non-functional heterodimer, which cannot bind to DNA; presumably, the mutant AhR exerts its dominant negative effect by competing with wild-type AhR. Expression of the dominant negative AhR mutant in wild-type cells reduced the extent to which TCDD down-regulates MHC Q1\textsuperscript{b} (Fig. 7); this finding implies that down-regulation of MHC Q1\textsuperscript{b} by TCDD requires AhR. This conclusion is consistent with our observation that the EC\textsubscript{50} for MHC Q1\textsuperscript{b} down-regulation is similar to that for a known AhR-dependent response, CYP1A1 induction. In addition, our conclusion is consistent with the Arnt dependence of MHC Q1\textsuperscript{b} down-regulation, because AhR-mediated responses also require Arnt.

Transcriptional and Post-transcriptional Regulation of MHC Q1\textsuperscript{b} Gene Expression by TCDD—Down-regulation of MHC Q1\textsuperscript{b} by TCDD could be due to a decrease in the rate of RNA synthesis, to an increase in the rate of RNA degradation, or to both. To determine whether TCDD regulates MHC Q1\textsuperscript{b} at the level of transcription, we performed nuclear run-on experiments. Multiple experiments (see Fig. 8 for example) reveal that TCDD consistently decreases the rate of MHC Q1\textsuperscript{b} transcription; however, we observed substantial variation in the extent of the decrease, ranging from 2- to 10-fold. This finding suggests that the down-regulation of MHC Q1\textsuperscript{b} by TCDD occurs, at least in part, at the transcriptional level.

We used actinomycin D to determine whether TCDD also has a post-transcriptional effect and influences the degradation rate of MHC Q1\textsuperscript{b} mRNA. When cells are exposed to actinomycin D at concentrations that inhibit transcription by \(95\%\) (26), MHC Q1\textsuperscript{b} mRNA remains elevated for at least 12 h (Fig. 9). This finding implies that, in the absence of TCDD, MHC Q1\textsuperscript{b} mRNA has a relatively long half-life, because its concentration does not fall when transcription is blocked. Therefore, because
MHC Q1b mRNA concentration falls in the presence of TCDD, we infer that TCDD must increase the rate of MHC Q1b mRNA degradation. We note that actinomycin D blocks the TCDD-induced decrease in MHC Q1b mRNA (Fig. 9). This result implies that TCDD acts indirectly to increase MHC Q1b mRNA degradation, because the effect requires ongoing RNA synthesis. Taken together, our studies imply that TCDD influences the regulation of MHC Q1b gene expression at both transcriptional and post-transcriptional levels.

Specificity of TCDD’s Effect on MHC Q1b Gene Expression—Because cells often express multiple forms of class I MHC molecules, we examined TCDD’s effect on the expression of MHC H-2Kb and H-2Db, two classical class I MHC genes, and on the expression of MHC Q10b, a nonclassical class I MHC gene. RNase protection studies with MHC-specific probes reveal that TCDD does not effect the expression of H-2Db (Fig. 10). We did not detect H-2Kb and Q10b mRNAs in these cells. These results indicate that TCDD does not down-regulate all class I MHC genes; its effect may be specific for MHC Q1b gene expression.

DISCUSSION

To generate a broader perspective on the biological effects of TCDD and to uncover novel mechanistic aspects of TCDD action, we used differential display to find new dioxin-responsive genes in mouse hepatoma cells. We isolated full-length cDNA for the TCDD-responsive MHC Q1b gene, and we analyzed the mechanism of its down-regulation.

Sequence analyses of MHC Q1b cDNA clones reveal high homology to the MHC Q1k gene. The analyses also imply the existence of several splicing variants. However, none of the MHC Q1b clones is spliced as predicted for MHC Q1k (22). Three of seven clones are spliced from exon 5 to exon 8, which deletes the protein’s cytoplasmic domain. Two clones contain intron 5, which produces an abnormal shortening of the cytoplasmic domain. Multiple spliced transcripts have been observed in studies of other nonclassical class I MHC mRNAs (27–29). Our observations extend these previous findings and suggest that multiple mRNA splicing may be common in nonclassical class I MHC gene expression.

Classical class I MHC proteins, which are encoded by the H-2 K, D, and L regions of mouse chromosome 17 (see Ref. 40 and references therein) present foreign peptides primarily to cytotoxic T cells, which lyse the cells that harbor the antigen (such as a viral peptide). In contrast, the function of nonclassical class I MHC proteins, which are encoded by the Q and T regions, is not well understood; these proteins may have di-
verse functions. For example, the product of the MHC Q1 gene is secreted (30, 31), whereas the product of the MHC Q5 gene is cytoplasmic (32). Therefore, MHC Q10 and Q5 are unlikely to be involved in antigen presentation to cells of the immune system. On the other hand, the nonclassical class I MHC T10 and T22 gene products may participate in presenting antigen to T cells with y receptors (33, 34). Our findings reveal that each MHC Q1 gene cDNA encodes the a3 domain and the transmembrane domain; these observations imply that the MHC Q1 gene product could interact with b2-microglobulin and could be a membrane protein. Therefore, we envision that MHC Q1 gene could function in antigen presentation; however, this hypothesis remains to be tested. We used RT-PCR to analyze MHC Q1 gene expression in tissues, and we detected Q1 mRNA in mouse liver, testis, kidney, lung, and spleen. Therefore, MHC Q1 might function in numerous tissues. TCDD elicits a variety of immunological effects in experimental animals, including suppression of both T cell-mediated immunity and humoral immunity (3). Down-regulation of a class I MHC gene(s) represents a direct mechanism by which TCDD could suppress T cell-mediated immunity, because it might adversely affect antigen presentation. For example, mice exposed to TCDD exhibit enhanced susceptibility to viral infection (35). We speculate that down-regulation of MHC gene expression by TCDD could hinder the presentation of viral peptides to cytotoxic T cells, thereby enhancing survival of the virus and increasing the susceptibility of the host to its adverse effects. The mechanism by which TCDD down-regulates gene expression has not been studied in detail. Our findings indicate that down-regulation of MHC Q1 mRNA is an AhR/Arnt-mediated response and occurs at both transcriptional and post-transcriptional levels. We can envision several possible mechanisms for transcriptional down-regulation: 1) the AhR/Arnt heterodimer could interact directly with a silencer-like element(s) (36) on the MHC Q1 gene; 2) AhR/Arnt could disrupt the DNA binding of a positive regulatory factor(s) by interacting at an overlapping AhR/Arnt recognition sequence (37); 3) AhR/Arnt could interact directly with another transcription factor(s), thereby squelching constitutive expression of MHC Q1; 4) AhR/Arnt could induce a gene whose product down-regulates MHC Q1 transcription (38). The identification of MHC Q1 transcriptional regulatory elements and analyses of protein-DNA interactions at these elements may enable us to test these hypotheses in the future. Our findings imply that post-transcriptional regulation must be important in TCDD-induced down-regulation of MHC Q1 gene expression, because MHC Q1 mRNA is stable in the absence of ongoing RNA synthesis. We note that the 3 untranslated region of MHC Q1 contains four adenylate-uridylate-rich elements (AUREs), AUAUU. Such elements can function as mRNA-destabilizing signals by an unknown mechanism.

2 L. Dong, Q. Ma, and J. P. Whitlock, Jr., unpublished observations.

REFERENCES
1. Poland, A., and Knutson, J. C. (1982) Annu. Rev. Pharmacol. Toxicol. 22, 517–554
2. Safe, S. H. (1986) Annu. Rev. Pharmacol. Toxicol. 26, 371–399
3. Holapple, M. P., Snyder, N. K., Wood, S. C., and Morris, D. L. (1991) Toxicology 69, 219–255
4. Whitlock, J. P., Jr. (1993) Chem. Res. Toxicol. 6, 754–763
5. Whitlock, J. P., Jr., Okino, S. T., Dong, L., Ko, H. P., Clarke-Katenberg, R., Ma, Q., and Li, H. (1996) FASEB J. 10, 809–818
6. Conney, A. H. (1982) Cancer Res. 42, 4875–4917
7. Swanson, H. I., and Bradfield, C. A. (1993) Pharmacogenetics 3, 213–230
8. Hanscom, O. (1995) Annu. Rev. Pharmacol. Toxicol. 35, 307–340
9. Okino, S. T., and Whitlock, J. P., Jr. (1995) Mol. Cell. Biol. 15, 3714–3721
10. Ko, H. P., Okino, S. T., Ma, Q., and Whitlock, J. P., Jr. (1996) Mol. Cell. Biol. 16, 430–436
11. Ko, H. P., Okino, S. T., Ma, Q., and Whitlock, J. P., Jr. (1997) Mol. Cell. Biol. 17, 3497–3507
12. Liang, P., and Pardee, A. B. (1992) Science 257, 967–971
13. Liang, P., Averboukh, L., Koyrmasov, K., Sager, R., and Pardee, A. B. (1992) Cancer Res. 52, 6966–6968
14. Miller, A. G., Israel, D., and Whitlock, J. P., Jr. (1983) J. Biol. Chem. 258, 3523–3527
15. Hankinson, O. (1983) Somat. Cell Genet. 9, 497–514
16. Dong, L., Ma, Q., and Whitlock, J. P., Jr. (1996) J. Biol. Chem. 271, 7942–7948
17. Li, H., Dong, L., and Whitlock, J. P., Jr. (1994) J. Biol. Chem. 269, 28098–28105
18. Weiss, E., Golden, L., Zakut, R., Meller, A., Fahrner, K., Kvist, S., and Flavell, R. A. (1983) EMBO J. 2, 453–462
19. Duran, L. W., Horton, R. M., Birschbach, C. W., Change-Miller, A., and Pease, L. R. (1989) J. Immunol. 142, 288–296
20. Meller, A. L., Weiss, E. H., Kress, M., Jay, G., and Flavell, R. A. (1984) Cell 36, 139–144
21. Ansell, P. M., Brent, R., Kingston, R. E., Deidam, D. E., Seidman, J. G., Smith, J. A., and Struhl, K. (eds) (1990) Current Protocols in Molecular Biology, pp. 4.10.1–4.10.8, Greene Publishing Associates and Wiley-Interscience, New York
22. Watts, S., Davis, A. C., Gant, B., Wheeler, C., Hill, L., and Goodnow, R. S. (1989) EMBO J. 8, 1749–1759
23. Hood, L., Steinmetz, M., and Malissen, B. (1983) Annu. Rev. Immunol. 1, 529–568
24. Flavell, R. A., Allen, H., Burkly, L. C., Sherman, D. H., Waneck, G. L., and Widera, G. (1986) Science 233, 437–443
25. Chen, Y.-T., Obata, Y., Stockert, E., and Old, L. J. (1986) J. Exp. Med. 162, 1134–1148
26. Durrin, L. K., and Whitlock, J. P., Jr. (1989) Mol. Cell. Biol. 9, 5733–5737
27. Waneck, G. L., Sherman, D. H., Calvin, S., Allen, H., and Flavell, R. A. (1987) J. Exp. Med. 165, 1358–1370
28. Cheroutre, H., Krechmer, J. M., Bronson, K., Hunt, S. W., III, Eights, P., Hool, L., and Nickerson, D. A. (1991) J. Immunol. 146, 3263–3272
29. Obata, Y., Stockert, E., Chen, Y.-T., Takahashi, T., and Old, L. J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3541–3545
30. Kress, M., Cosman, D., Khein, G., and Jay, G. (1983) Cell 34, 189–196
31. Devinil, J. J., Lew, A. M., Flavell, R. A., and Coligan, J. E. (1985) EMBO J. 4, 369–374
32. Reyes-Rangel, A., Le Roy, E., Diegues, J. L., and Fernandez, N. (1993) J. Reprod. Immunol. 23, 73–81
33. Schild, H., Mavaddat, N., Litzenberger, C., Ehrich, E. W., Davis, M. M., Bluestone, J. A., Matis, L, Draper, R. K., and Chien, Y.-H. (1994) Cell 76, 29–37
34. Moriwaki, S., Korn, B. S., Ishikawa, Y., Van Kaer, L., and Tonegawa, S. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11396–11400
35. Thiggpen, J. E., Faith, R. E., McConnell, E. E., and Moore, J. A. (1975) Infect. Immun. 12, 1319–1324
36. Murphy, C., Nikodem, D., Howcroft, K., Weissman, J. D., and Singer, D. S. (1996) J. Biol. Chem. 271, 30992–30999
37. Krishnan, V., Porter, W., Santostefano, M., Wang, X., and Safe, S. (1995) Mol. Cell. Biol. 15, 6710–6719
38. Lee, D. C., Barlow, K. D., and Gaido, K. W. (1996) Toxicol. Appl. Pharmacol. 139–144
39. Rose, J. (1995) Microbiol. Rev. 59, 423–450
40. Kalicyanurumal, A., Falchetto, R., Cox, A., Dick, R., II, Shabanowitz, J., Chien, Y., Matis, L., Hunt, D. F., and Bluestone, J. A. (1995) J. Immunol. 155, 2379–2386