Organization and Expression of the Immunoresponsive Lysozyme Gene in the Giant Silk Moth, Hyalophora cecropia*

(Received for publication, October 16, 1990)

Shao-Cong Sun, Bengt Åsling, and Ingrid Faye

From the Department of Microbiology, University of Stockholm, S-106 91 Stockholm, Sweden

Lysozyme is one of the antibacterial proteins that are produced by the giant silk moth Hyalophora cecropia in response to bacterial infection or injury. As an essential step toward the understanding of the mechanisms involved in the immune response, we have isolated and characterized the lysozyme gene from Cecropia. The complete nucleotide sequence of the gene as well as the immediate flanking sequences have been determined. The gene includes three exons. Its first intron contains a repetitive sequence. In the evolutionary aspect, the Cecropia lysozyme gene and two vertebrate lysozyme genes have been found to maintain a similar organization pattern of exons. The lysozyme gene has been found to be strongly induced by lipopolysaccharides and a phorbol ester as well as bacteria. In the induction by bacteria, the lysozyme transcript appears about 2 h, reaches to the maximum level at about 24 h, and then declines. Comparison of the S'-flanking sequences with several other genes involved in the immune response of H. cecropia and Drosophila melanogaster revealed a C-like consensus sequence. This sequence is specifically recognized by a nuclear protein from the induced pupa.

Lysozyme (EC 3.2.1.17) is a widely distributed enzyme found in bacteriophages, microbes, plants, invertebrates, and vertebrates. Since its discovery in 1922, lysozyme has been extensively studied and used as a model system for investigating the three-dimensional structure of proteins, mechanism of enzyme action, and molecular evolution (reviewed in Jollès and Jollès, 1984). Amino acid sequence data on lysozymes are available from a large number of vertebrate species (Jollès and Jollès, 1984). Lysozyme cDNAs from chicken, mouse, cow, deer, pig, and human have been cloned and sequenced (Jung et al., 1988). The complete nucleotide sequence of the gene as well as bacteria. In the induction by bacteria, the lysozyme transcript appears at about 2 h, reaches to the maximum level at about 24 h, and then declines. Comparison of the S'-flanking sequences with several other genes involved in the immune response of H. cecropia and Drosophila melanogaster revealed a C-like consensus sequence. This sequence is specifically recognized by a nuclear protein from the induced pupa.

Lysozyme (EC 3.2.1.17) is a widely distributed enzyme found in bacteriophages, microbes, plants, invertebrates, and vertebrates. Since its discovery in 1922, lysozyme has been extensively studied and used as a model system for investigating the three-dimensional structure of proteins, mechanism of enzyme action, and molecular evolution (reviewed in Jollès and Jollès, 1984). Amino acid sequence data on lysozymes are available from a large number of vertebrate species (Jollès and Jollès, 1984). Lysozyme cDNAs from chicken, mouse, cow, deer, pig, and human have been cloned and sequenced (Jung et al., 1988; Cross et al., 1988; Irwin and Wilson, 1989; Irwin and Wilson, 1990; Li et al., 1988). Genomic sequences of the chicken lysozyme gene and the mouse lysozyme M gene have also been determined (Jung et al., 1988; Cross et al., 1988). The vertebrate lysozymes, on the other hand, are less studied. Although several lysozymes have been identified and isolated from insects (Powning and Davidson, 1973; Powning and Davidson, 1976; Schneider, 1985; Hultmark et al., 1980; Jollès et al., 1979), information on their primary structures is lacking in many cases. So far, the best studied insect lysozyme is the one from the giant silk moth Hyalophora cecropia (Engström et al., 1985). Lysozyme, together with cecropins and attacins, are the three main types of antibacterial proteins induced in the diapausing pupae of Cecropia by bacterial infection or injury. They constitute the major antibacterial components of the humoral immune system in Cecropia (Boman and Hultmark, 1987). As lysozyme is bactericidal only to a few Gram-positive bacteria that are also sensitive to cecropins, it is believed that the main function of lysozyme is to remove the murein sacculus left over after the action of cecropins and attacins (Hultmark et al., 1980; Boman and Hultmark, 1987). The Cecropia lysozyme has been isolated (Hultmark et al., 1980), and the protein sequence as well as the nucleotide sequence of its cDNA have been determined (Engström et al., 1985). It shows great sequence similarity to the chicken-type lysozymes.

We are interested in the regulation of the expression of the immune genes in Cecropia. As an essential step toward this goal, we have analyzed the structures of a number of immune genes from Cecropia. Here, we report the isolation, sequence characterization, and expression of the Cecropia lysozyme gene. A tentative promoter element will be discussed.

MATERIALS AND METHODS

Insects and Injection—Dispausing pupae of Cecropia were either purchased from American dealers or reared on a synthetic diet (Hultmark et al., 1980) and stored at 8°C. The pupae were injected with either Enterobacter cloacae β12 (Hultmark et al., 1980), 100 μg of lipopolysaccharide (LPS)1 from Escherichia coli D21 in Lepidopteran Ringer or 300 ng of phorbol 12-myristate 13-acetate (PMA) (dissolved in dimethyl sulfoxide and diluted with Lepidopteran Ringer just before use) in a volume of 50 μl. As a control, 50 μl of Lepidopteran Ringer containing the same amount of dimethyl sulfoxide as the PMA solution was injected.

Isolation of Cecropia Lysozyme Genomic Clones—A Cecropia genomic bank in the λEMBL 3 vector was constructed from a single pupa.2 It was screened with the Cecropia lysozyme cDNA, pCP701 (Engström et al., 1985), 32P-labeled to high specific activity by random priming using an oligolabeling kit (Pharmacia LKB Biotechnology Inc.). Membrane transfer and hybridization, using Du Pont-New England Nuclear's NEF filters, were carried out according to the manufacturer's instructions (Du Pont). Phage DNA was isolated essentially according to Garber and Kuroiwa (1983), using E. coli Q54S as host bacteria. Restriction mapping and Southern blot hybridizations (Southern, 1975) were used to analyze the cloned DNA.

Cecropia DNA Preparation—The tissue of Cecropia pupae, mainly containing the fat body, was prepared as described (Xanthopoulos et al., 1988) and ground in a liquid nitrogen-containing mortar. The preparation of nuclei and subsequent proteinase K digestion were carried out essentially according to Fritton et al. (1983). After phenol extraction, DNA was ethanol precipitated and treated according to standard procedures. Following precipitation, DNA was redissolved in sterile 10 mM Tris-HCl, pH 8.0, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1% sodium dodecyl sulfate, and 0.5 mg/ml of proteinase K was added. The DNA was kept at 37°C for 60 min, followed by phenol extraction and ethanol precipitation.

1 The abbreviations used are: LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; kb, kilobase pair(s); bp, base pair(s).
2 G. Gudmundsson, D.-A. Lindholm, B. Åsling, R. Gan, and H. G. Boman, personal communication.
Southern blot analysis of Cecropia genomic DNA—Cecropia chromosomal DNA was digested with restriction enzyme EcoRI, blotted, and probed with the Cecropia lysozyme cDNA. The cloned DNA, XCP701, was used as a control (lane G). The positions of the λ size markers are indicated. The digests of samples from pupae A, B, and C were repeated and run in lanes A', B', and C', respectively. B, the DNA filter in A was probed with a genomic DNA fragment covering 866 bases from the 3' end of the first exon.

Cecropia lysozyme cDNA. Fig. 2A shows two independent digestions of DNA from the three individual pupae (A—C and A'—C'). All three pupae show a new hybridizing EcoRI fragment in the same size range as the hybridizing EcoRI fragment from the genomic clone ΧCP701 (lane G). The weak band seen in the DNA digests from pupae C is most likely due to partial digestions since its strength, as compared with the main band, is different in the two digestes. We then conclude that the Cecropia genome contain a single lysozyme gene.

The small size discrepancies of the EcoRI fragment from the individual pupae suggested a restriction site polymorphism. This was confirmed by additional genomic Southern blot analysis (data not shown), and the size difference was localized to the XhoI-EcoRI fragment that contains the 3'-flanking region (Fig. 1A).

Sequence Characteristics of the Lysozyme Gene—Since ΧCP701 contains the complete lysozyme gene, this clone was selected for further analysis. As the first step of subcloning, the larger EcoRI fragment from clone ΧCP701 was inserted

FIG. 1. The organization of the Cecropia lysozyme gene. A, restriction map of the Cecropia lysozyme gene locus. B, the overlapping Cecropia lysozyme genomic clones in the λEMBL3 vector. The direction of transcription of the lysozyme gene is indicated by an arrow. C, sequencing strategy for the lysozyme gene. The arrows indicate the direction and length of individual sequencing experiments. The subclones in the sequencing were obtained either by the shot gun method (○) or by deletion (●).
into pT7Z19R. Further subcloning and sequencing were carried out as described under "Materials and Methods." The sequencing strategy is shown in Fig. 1C. In most cases single-stranded templates were used, and the sequencing was done on both strands. When only one strand was sequenced, the sequencing experiment was repeated at least twice or until no uncertainties were left.

The sequence of the Cecropia lysozyme genes, together with the 5'-flanking region, is shown in Fig. 3. The whole transcriptional unit of the gene (from cap site to the second polyadenylation site) is 2875 bp long. The gene contains three exons interrupted by two introns with the boundary sequences of 5' ACTgtgaa—cagGG 3' (intron 1) and 5' ATCgtaaggt—tagAG 3' (intron 2), which are in agreement with the consensus intron/exon border sequences of eukaryotic genes (Breathnach and Chambon, 1981). The first intron is much larger than the second one, 1644 as compared with 668 bp. A further analysis of the first intron is discussed below.

An insect specific cap site with the consensus sequence ATCA,TTPy (Snyder et al., 1982; Hultmark et al., 1986) has earlier been found in the cercopin B gene from Cecropia (Xanthopoulos et al., 1988). In the Cecropia lysozyme gene, we found a similar heptamer sequence, ATCATAC, 49 bases downstream from the cap site. This sequence homologous to TATA box (Breathnach and Chambon, 1981). The first intron is much larger than the second one, 1644 as compared with 668 bp. A further analysis of the first intron is discussed below.

The Cecropia Lysozyme Gene Contains a Repetitive Sequence in its First Intron—Repetitive sequences have been found in some vertebrate lysozyme genes (Li et al., 1988; Baldacci et al., 1981) and lysozyme-related genes (Hall et al., 1987). A transposable element has also been identified from the intron of the cercopin A gene in Cecropia. We investigated the possibility of a transposable element in the Cecropia lysozyme gene. The DNA filters used for the genomic Southern blots were re-probed with various subclones of ACP701. One of the subclones covering 1056 bases of the first intron and 74 bases of the second exon was shown to give a repetitive pattern. Deletion of the exon part and another 190 bases of the intron sequence from this subclone slightly changed its hybridization pattern but did not destroy its repetitive property (Fig. 2B), whereas further deletion of about 380 bases from the same direction totally destroyed its ability to generate a repetitive hybridization pattern. The repetitive DNA sequence is localized in a region of 570 bases at the 3' end of the intron (Fig. 3, sequence in brackets).

Determination of the Transcriptional Initiation Site in Injured and Immune Cepae—As mentioned above, the Cecropia lysozyme gene at its 5'-flanking region has a heptamer sequence homologous to the cap site consensus sequence of the insect genes. To see if this sequence is used as an initiation site in the transcription of the lysozyme gene, we carried out a RNase protection assay. A 566-bp DNA fragment was transcribed in vitro to generate a radioactive RNA probe which could protect a 122-bp nucleotide fragment counting from the heptamer sequence. RNA from immune (16 h after bacterial injection), injured (16 h after Lepidopteran Ringer injection), or noninjected pupae were used. As shown in Fig. 4, two bands (marked by arrows) resulted from the protection for the immune RNA (lane 2) and the injured RNA (lane 3). The major band, with the size of about 122 bp, is in agreement with the expected size, indicating the presence of a lysozyme transcript initiated from the heptamer. The RNase protection also showed that the injury response was much weaker than the immune response and that the same sites for the initiation of transcription were used.

The kinetics of bacterial induction of lysozyme mRNA—

3 D.A. Lindholm, G. H. Gudmundsson, and H. G. Boman, personal communication.
The Cecropia lysozyme mRNA can be induced by LPS and a Phorbol Ester (PMA)—It has been reported that bacterial LPS and phorbol esters activate a number of mammalian genes containing a β-like promoter element via the activation of a transcription factor, NF-κB (Lenardo and Baltimore, 1989). Since the Cecropia lysozyme gene also contains a β-like sequence element at the promoter region, we investigated the effect of LPS and PMA on the expression of this gene. The Cecropia pupae were injected with LPS, PMA, or Lepidopteran Ringer as described under “Materials and Methods.” The induction of lysozyme mRNA was followed by RNase protection assay. As shown in Fig. 5B, a low level of lysozyme mRNA was induced by the injection of Lepidopteran Ringer, indicating the wound response. When injected with as 4 h and declined to a very low level at 16 h (see also Fig. 4). This band declined and almost disappeared 24 h after injection. From this result we conclude that there are two alternative initiation sites for transcription (Fig. 6).

The Cecropia Lysozyme mRNA Can Be Induced by LPS and a Phorbol Ester (PMA)—It has been reported that bacterial LPS and phorbol esters activate a number of mammalian genes containing a β-like promoter element via the activation of a transcription factor, NF-κB (Lenardo and Baltimore, 1989). Since the Cecropia lysozyme gene also contains a β-like sequence element at the promoter region, we investigated the effect of LPS and PMA on the expression of this gene.

The Cecropia pupae were injected with LPS, PMA, or Lepidopeteran Ringer as described under “Materials and Methods.” The induction of lysozyme mRNA was followed by RNase protection assay. As shown in Fig. 5B, a low level of lysozyme mRNA was induced by the injection of Lepidopteran Ringer, indicating the wound response. When injected with

The induction kinetics of lysozyme gene expression was carried out using Cecropia diapausing pupae. The pupae were injected with E. cloacae β12 and used for RNA preparation at different time intervals after injection. The induction of the lysozyme RNA was detected by both Northern blot (data not shown) and RNase protection analyses (Fig. 5A). Immediately after injection (0 h) of bacteria, no detectable lysozyme transcript was found. However, 2 h after injection a small amount of lysozyme RNA appeared. Like in the first RNase protection assay (Fig. 4), we could see two transcripts. They showed different kinetics in the bacterial induction. The larger transcript reached the maximum level at about 24 h and then declined. The smaller one reached the maximum level as early

The induction kinetics of lysozyme gene expression was carried out using Cecropia diapausing pupae. The pupae were injected with E. cloacae β12 and used for RNA preparation at different time intervals after injection. The induction of the lysozyme RNA was detected by both Northern blot (data not shown) and RNase protection analyses (Fig. 5A). Immediately after injection (0 h) of bacteria, no detectable lysozyme transcript was found. However, 2 h after injection a small amount of lysozyme RNA appeared. Like in the first RNase protection assay (Fig. 4), we could see two transcripts. They showed different kinetics in the bacterial induction. The larger transcript reached the maximum level at about 24 h and then declined. The smaller one reached the maximum level as early

The Cecropia Lysozyme mRNA Can Be Induced by LPS and a Phorbol Ester (PMA)—It has been reported that bacterial LPS and phorbol esters activate a number of mammalian genes containing a β-like promoter element via the activation of a transcription factor, NF-κB (Lenardo and Baltimore, 1989). Since the Cecropia lysozyme gene also contains a β-like sequence element at the promoter region, we investigated the effect of LPS and PMA on the expression of this gene.

The Cecropia pupae were injected with LPS, PMA, or Lepidopteran Ringer as described under “Materials and Methods.” The induction of lysozyme mRNA was followed by RNase protection assay. As shown in Fig. 5B, a low level of lysozyme mRNA was induced by the injection of Lepidopteran Ringer, indicating the wound response. When injected with

The Cecropia Lysozyme mRNA Can Be Induced by LPS and a Phorbol Ester (PMA)—It has been reported that bacterial LPS and phorbol esters activate a number of mammalian genes containing a β-like promoter element via the activation of a transcription factor, NF-κB (Lenardo and Baltimore, 1989). Since the Cecropia lysozyme gene also contains a β-like sequence element at the promoter region, we investigated the effect of LPS and PMA on the expression of this gene.

The Cecropia pupae were injected with LPS, PMA, or Lepidopteran Ringer as described under “Materials and Methods.” The induction of lysozyme mRNA was followed by RNase protection assay. As shown in Fig. 5B, a low level of lysozyme mRNA was induced by the injection of Lepidopteran Ringer, indicating the wound response. When injected with

The induction kinetics of lysozyme gene expression was carried out using Cecropia diapausing pupae. The pupae were injected with E. cloacae β12 and used for RNA preparation at different time intervals after injection. The induction of the lysozyme RNA was detected by both Northern blot (data not shown) and RNase protection analyses (Fig. 5A). Immediately after injection (0 h) of bacteria, no detectable lysozyme transcript was found. However, 2 h after injection a small amount of lysozyme RNA appeared. Like in the first RNase protection assay (Fig. 4), we could see two transcripts. They showed different kinetics in the bacterial induction. The larger transcript reached the maximum level at about 24 h and then declined. The smaller one reached the maximum level as early
A Nuclear Protein Specifically Binds to the \( \kappa B \)-like Sequence of the Lysozyme Gene—We investigated the possible function of the \( \kappa B \)-like element in the regulation of the lysozyme gene using the DNA mobility shift assay. By RsaI restriction cleavages and exonuclease III deletions, we created two probes, \( P_{200} \) and \( P_{185} \), from the promoter region of the lysozyme gene. \( P_{200} \) contains the complete \( \kappa B \)-like sequence, whereas \( P_{185} \) has the major part of this site deleted (Fig. 6A). In the binding assay, nuclear extracts from both normal and bacteria-induced pupae were used. As shown in Fig. 6B, \( P_{200} \) could form a complex with the nuclear extract from bacteria-induced pupa (lane 3) but not with that from the normal pupa (lane 2). Furthermore, this binding could be abolished by using 30-fold of the unlabeled \( P_{200} \) (lane 4). However, \( P_{185} \) with the \( \kappa B \)-like sequence deleted was ineffective as a competitor even at a 50-fold molar excess (lane 5). The inability of \( P_{185} \) for this binding was further confirmed by using the end-labeled \( P_{183} \) directly in the binding assay (lane 6).

**DISCUSSION**

Amino acid heterogeneities in the Cecropia lysozyme were earlier found and explained as caused by allelic variants of the gene (Engström et al., 1985). Sequence comparison of the Cecropia lysozyme cDNA (Engström et al., 1985) with the Cecropia lysozyme gene reveals 2 base pair substitutions in the internal coding sequence. Both substitutions cause amino acid changes, Arg-15 (number concerning the mature protein) to Leu-15 and Thr-66 to Ser-66, that correspond to the discrepancies found previously.

The cDNA study demonstrated the similarity in the primary structure of the Cecropia and the chicken lysozymes (Engström et al., 1985). It is now possible to make a comparison of all the functional domains in the Cecropia lysozyme with those in two vertebrate lysozymes. In Fig. 7, we have aligned the full amino acid sequences deduced from the lysozyme gene (Engstrom et al., 1985) with the published sequences for the cecropin genes in Cecropia and Drosophila (Xanthopoulos et al., 1988). Kylsten et al. (1990) also revealed this type of element (Sun et al., 1991). Since the lysozyme is induced simultaneously with the attacins and the cecropins, we looked for this element in the upstream region of the lysozyme gene. We found a palindromic sequence, GGAGGATTCCCT, which is also homologous to the NF-\( \kappa B \) binding site. DNA mobility shift assay clearly showed that this \( \kappa B \)-like sequence could be specifically bound by a nuclear protein from the bacteria-induced Cecropia pupa. Deletion of the \( \kappa B \)-like sequence from the probe abolished this binding. Nuclear extract from the uninduced pupa did not show any binding activity. This result is consistent with the in vivo expression of the gene.

The \( \kappa B \) site was first found in the immunoglobulin \( \kappa \) gene and later in a variety of other genes. These genes can be induced by LPS, phorbol esters, and a number of other inducers via the activation of the NF-\( \kappa B \), which is normally present in the cytoplasm as an inactive form (reviewed in Lenardo and Baltimore, 1989). It has been shown earlier that LPS strongly induces the immune system in Cecropia (Trenzcek and Faye, 1988). We have now investigated the effect of LPS and a phorbol ester (PMA) on the expression of the lysozyme gene as well as the attacin genes that also contain the \( \kappa B \)-like promoter element. We found that these genes were strongly induced by both LPS and PMA (Fig. 5B) (Sun et al., 1991). Taken together with the earlier findings that the immune genes in insects are co-induced (Boman and Hultmark, 1987; Kylsten et al., 1990), these data indicate that a common trans-acting factor is involved in the induction of these immune genes. This factor shares certain similarities with NF-\( \kappa B \) probably in the way of activation and DNA binding specificity. Recently, NF-\( \kappa B \) was found to have the identical DNA binding subunit as KBF1, a factor recognizing the \( \kappa B \)-homologous sequence in major histocompatibility complex class 1 gene. Furthermore, these two factors were suggested to belong to the family of proteins that includes rel oncogenes and the Drosophila dorsal gene product (Kieran et al., 1990). The fact that the nuclear factor we found from Cecropia binds to a target sequence homologous to \( \kappa B \) (and also to the target of KBF1) raises the question whether it
represents another member of this \textit{rel} family.

\textbf{Acknowledgments}—We thank Gudmundur Gudmundsson for the genomic library. We also thank Hans G. Boman and Deborah Kimbrell for critical reading of the manuscript.

\textbf{REFERENCES}

Baldacci, P., Royal, A., Breggere, F., Abastado, J. P., Cami, B., Daniel, F., and Kourilsky, P. (1981) \textit{Nucleic Acids Res.} \textbf{9}, 3576-3588

Boman, H. G., and Hultmark, D. (1987) \textit{Annu. Rev. Microbiol.} \textbf{41}, 103-126

Breathnach, R., and Chambon, P. (1981) \textit{Nucleic Acids Res.} \textbf{9}, 3575-3588

Boman, H. G., and Hultmark, D. (1987) \textit{Annu. Rev. Microbiol.} \textbf{41}, 103-126

Breathnach, R., and Chambon, P. (1981) \textit{Annu. Rev. Biochem.} \textbf{50}, 349-383

Baldacci, P., Royal, A., Breggere, F., Abastado, J. P., Cami, B., Daniel, F., and Kourilsky, P. (1981) \textit{Nucleic Acids Res.} \textbf{9}, 3575-3588

Boman, H. G., and Hultmark, D. (1987) \textit{Annu. Rev. Microbiol.} \textbf{41}, 103-126

Breathnach, R., and Chambon, P. (1981) \textit{Annu. Rev. Biochem.} \textbf{50}, 349-383

Cross, M., Mangelsdorf, I., Wedel, A., and Renkawitz, R. (1988) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{85}, 6232-6236

Efstratiadis, A., Posakony, J. W., Maniatis, T., Lawn, R. M., O’Connell, C., Spritz, R. A., Forget, B. G., Weissman, S. M., Slightom, J. L., Blechl, A. E., Smithies, O., Baralle, F. E., Shoulders, C. C., and Proudfoot, N. J. (1980) \textit{Cell} \textbf{21}, 653-668

Engstrom, A., Xanthopoulos, K. G., Boman, H. G., and Bennich, H. (1985) \textit{EMBO J.} \textbf{4}, 2119-2122

Fritton, H. P., Sippel, A. E., and Igo-Kemenes, T. (1983) \textit{Nucleic Acids Res.} \textbf{11}, 3467-3485

Garber, R. L., Kuroiwa, A., and Gehring, W. J. (1983) \textit{EMBO J.} \textbf{11}, 2027-2036

Gilbert, W. (1978) \textit{Nature} \textbf{271}, 501

Gilman, M. (1987) in \textit{Current Protocols in Molecular Biology} (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds) pp. 471-478, John Wiley & Sons, New York

Gorski, K., Carneiro, M., and Schibler, U. (1986) \textit{Cell} \textbf{47}, 767-776

Hall, L., Emery, D. C., Davies, M. S., Parker, D., and Craig, R. K. (1987) \textit{Biochem. J.} \textbf{242}, 735-742

Hultmark, D., Steiner, H., Raemdonc, T., and Boman, H. G. (1980) \textit{Eur. J. Biochem.} \textbf{106}, 7-16

Hultmark, D., Klemenz, R., and Gehring, W. J. (1986) \textit{Cell} \textbf{44}, 429-438

Irwin, D. M., and Wilson, C. A. (1989) \textit{J. Biol. Chem.} \textbf{264}, 11387-11393

Irwin, D. M., and Wilson, C. A. (1990) \textit{J. Biol. Chem.} \textbf{265}, 4944-4952

Jollés, P., and Jollés, J. (1984) \textit{Mol. Cell. Biochem.} \textbf{63}, 165-189

Jollès, J., Schoentgen, F., Croizier, G., Croizier, L., and Jollès, P. (1979) \textit{J. Mol. Biol.} \textbf{14}, 267-271

Jung, A., Sippel, E. A., Grez, M., and Schütz, G. (1980) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{77}, 5759-5763

Kieran, M., Blank, V., Logeat, F., Vandeveerkhove, J., Lottspeich, F., Le Bail, O., Urban, M. B., Kourilsky, P., Basuerle, P. A., and Israel, A. (1990) \textit{Cell} \textbf{62}, 1007-1018

Klemenz, R., Hultmark, D., and Gehring, W. J. (1985) \textit{EMBO J.} \textbf{4}, 2053-2060

Kylsten, P., Samakovlis, C., and Hultmark, D. (1990) \textit{EMBO J.} \textbf{9}, 217-224

Lenardo, M. J., and Baltimore, D. (1989) \textit{Cell} \textbf{58}, 227-229

Li, P.-C., Keshav, S., and Gordon, S. (1988) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{85}, 6227-6231

Maniatis, T., Pritsch, E. F., and Sambrook, J. (1982) \textit{Molecular Cloning: A Laboratory Manual}, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

McGinnis, W., Levine, M. S., Hafen, E., Kuroiwa, A., and Gehring, W. J. (1984) \textit{Nature} \textbf{308}, 428-433

Powning, R. F., and Davidson, W. J. (1973) \textit{Comp. Biochem. Physiol.} \textbf{45B}, 669-681

Powning, R. F., and Davidson, W. J. (1976) \textit{Comp. Biochem. Physiol.} \textbf{55B}, 221-228

Powning, R. F., and Davidson, W. J. in press

Southern, E. M. (1975) \textit{J. Mol. Biol.} \textbf{98}, 503-517

Sanger, F., Miklen, S., and Coulson, A. R. (1977) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{74}, 5463-5467

Schneider, P. M. (1985) \textit{Insect Biochem.} \textbf{15}, 463-470

Snyder, M., Hunkapiller, M., Yuen, D., Silver, D., Fristrom, J., and Davidson, N. (1982) \textit{Cell} \textbf{29}, 1027-1040

Sun, S.-C., Lindstrom, I., Lee, J.-Y., and Faye, I. (1991) \textit{Eur. J. Biochem.}, in press

Trenczek, T., and Faye, I. (1988) \textit{Insect Biochem.} \textbf{18}, 299-312

Xanthopoulos, K. G., Lee, J.-Y., Gan, R., Kockum, K., Faye, I., and Boman, H. G. (1988) \textit{Eur. J. Biochem.} \textbf{172}, 371-376