Researches and Progresses of Modern Technology on Silk, Textile and Mechanicals

(I)

The 6th China International Silk Conference
and
The 2nd International Textile Forum

Suzhou, Jiangsu, P. R. China
Sep. 13-14, 2007

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Soochow University
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Chemical Industry Press

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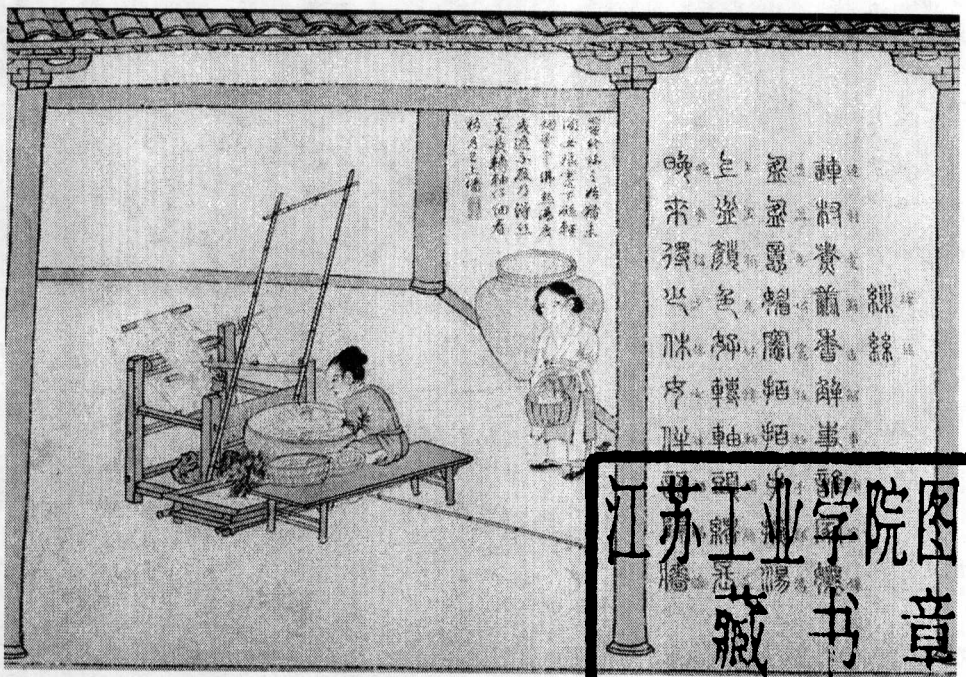

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Preface

The China International Silk Conference was hold successfully in 1991, 1993, 1996, 2000 and 2004 respectively. It has made great contribution for the development as well as the international exchange and cooperation of world silk industries. In 21 century, modern science and technology influence our society and lifestyle. What kind of prospects for the mulberry and silk industries we can expect? How to bring the silk products, which used to be the treasure of the human civilization into the new century? How to apply the hi-tech into the silk industries? All the people who love the silk will concern and think these questions. The 6th China International Silk Conference provides the platform for us to discuss these themes.

The 2nd international Textile Forum is jointly organized by The University of Manchester, UK, North Carolina State University, USA, Shinshu University, Japan, The Hong Kong Polytechnic University and Soochow University, China. The 1st International Textile Forum was held successfully at University of Manchester in 2006. It set the agenda for higher education and research in the 21st century. The forum offered a platform for information exchanges among researchers, academics, industrialists and retailers. The 2nd International Textile Forum will hold at Soochow University, China together with the China International Silk Conference. It provides the opportunity for textile professionals and academics of China to join the international teams of textile science and technology. It will also record the history of the development of high education of textile in China.

The China International Silk Conference and International Textile Forum will be hold at September 13 – 14 2007 in Soochow University. More than 200 papers were sent to the conference organizing committee. 105 papers are incorporated in this proceedings based on the results of specialists approval. The two volume proceedings are edited by Soochow University and published by Chemical Industry Press. Many specialists give great supporting, and more than 10 textile educationalists worked for the publication of the proceedings. Their support and work will be highly appreciated.

Editing Committee of Conference Proceedings

Aug. 30, 2007

CONTENT

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VII 其它

Role of IAP Gene of *Bombyx mori* in the Process of Baculovirus Infection

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Abstract: Inhibition of apoptosis protein (iap) factor was amplificated from silkworm using RT-PCR. A recombinant baculovirus, carrying *iap* gene of *Bombyx mori* was successfully constructed for the large-scale production of IAP protein by Bac-to-Bac system. DNA of the recombinant baculovirus was transfected into Bm cells of the silkworm recombinant virus. Cell morphology was observed and cell survival rate was calculated at different time post infection. The results showed that the *iap* gene possessed anti-apoptotic role in the process of baculovirus infection. Over-expression of the *iap* gene at host cells could extend the life span and life cycle of virus.

1. Introduction

Apoptosis or programmed cell death is a cellular suicide process in which damaged or harmful cells are eliminated from multicellular organisms [1]. Apoptosis plays an important role in the development and homeostasis of metazoans [2] and is also critical in insect embryonic development and metamorphosis [3,4]. Furthermore, apoptosis acts as a host defense mechanism by which virally infected cells are eliminated to limit the propagation of viruses [5].

Inhibitor of apoptosis proteins (IAPs) were originally discovered in baculoviruses [6,7]. IAPs were discovered in recent years for a new kind of cell apoptosis inhibitory protein, which is widely existed in many species. Molecular mechanisms of apoptosis showed that IAP is a very important and conservative protein family. So far, two baculovirus IAP, seven mammals IAP, three Arthropod IAP and Drosophila IAP were reported. However, there are few studies on the anti-apoptosis role of Bombyx mori IAP in the process of baculovirus infection of the cells of silkworm.

2. Materials

2.1. Cloning of BmIAP

Total RNA of BmN cells was extracted using RNA extraction:TRIZOL Reagent (Invitrogen , USA) according to the manufacturer's instructions. The total RNAs were treated with RNase-free DNase I (Gibco BRL, USA) in order to remove any contaminating genomic DNA before reverse transcription (RT). The PCR primers specifically for amplification of IAP gene were designed according to the sequence published in GenBank (Accession No. AF448494). The primers were:

F-GAAGGATCCATGGAGTTGACGAAAGTT;

R-GAAGAATTCTCACGAGAAGTAGAGCG.

2.2. Cell culture

Bombyx mori cells were cultured at 27°C in TC-100 media with 10% FBS. The cells served as the in vitro host for construction of the recombinant virus and virus titer.

2.3. Construction of recombinant transfect vector for B. mori iap gene

The vector pFastBacHTb-BmIAP was constructed by inserting the appropriate BmIAP fragment with two

sites of endonuclease BamH I and EcoR I. After ligation, the plasmids were tranfered into E. coli Jm101 for selection of recombinant transfect vector. The plasmids were extracted from the clones to determine the positive clone by digestion of endonuclease BamH I and EcoR I. The recombinant plasmids were transferred into E. coli BM-DH10 (A gift from Dr. Park EK, Japan)

2.4. Analysis of Recombinant Bacmid

Recombinant bacmid DNA is greater than 135 kilo base pairs in size. Since restriction analysis is difficult to perform with DNA of this size, we recommend using PCR analysis to verify the presence of the gene in the recombinant bacmid. The bacmid contains M13 Forward (-40) and M13 Reverse priming sites flanking the mini-attTn7 site within the lacZ α -complementation region to facilitate PCR analysis (as showed in following figure). Guidelines and instructions are provided in this section to perform PCR using the M13 Forward (-40) and M13 Reverse primers, the sequence are as follow:

F-CGCCAGGGTTTTCCCAGTCACGAC;

R- AGCGGATAACAATTTCACACGGGA.

2.5. Producing Recombinant Baculovirus Transfecting Insect Cells

Recombinant bacmid DNA was transfected insect cells with Cellfectin Reagent (Invitrogen) . Cells showed lesions after inoculation with the virus-containing supernatant resume growth of the previous few insect cells. The cells were collected and lyzed by heating as the template to detect recombinant virus, which named as r- IAP.

2.6. Morphological changes observed under the microscope

The recombinant virus r- IAP DNA transfected into silkworm cells and the budding virus was obtained. After several infection cycles, the titer of the recombinant virus was determined. Morphological changes of the cells were observed under microscopy at 24h, 48h, 72h, 96h, 108h post infection, respectively.

2.7. Determination of cell survival

Cell survival was determined by 0.4 % trypan blue staining (1:1 volume) of 2 to 3 min, using blood cell count plate. Dead cells can absorb trypan blue and showed blue, live cells were stained colorless and were transparent. Cell viability is the percentage of number of the living cells dividing the total number of the cells.

The virus infected the BmN cells with the multiple of infection as 1, the BmPAK6 virus as a control. The cell survival was counted at every 24 h post infection. The experiment was repeated three times, each sample was selected for three counts of vision.

3. Results and Discussion

3.1. The amplification of Bm-iap gene

A specific DNA fragment was amplified with the size of 1041bp, which was speculated encoding 293 amino acids with a molecular weight of 38.8 kDa (as showed in Fig. 1).

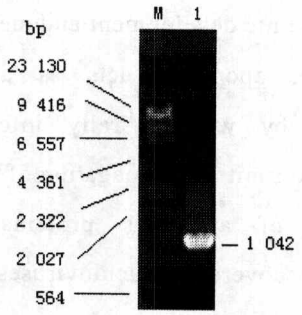

Fig. 1 The result of RT-PCR of *iap* gene from *B. mori*
M, DNA molecular weight marker;
1, PCR products of *iap* gene

3.2. The Construction of recombinant transfer vector for B.mori IAP *gene*

The recombinant plasmid was identified by digestion of *Eco*R I and *Bam*H I. Electrophoresis analysis showed a vector fragment with 4.8 kbp and an insert fragment of IAP PCR product. It confirmed

that pFastBacHTb-*iap* was positive recombinant plasmid (Figure 2 and 3).

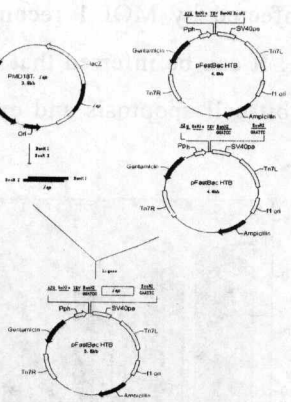

Fig.2 Consturction of recombinant plasmid pFastBacHTb-*iap*

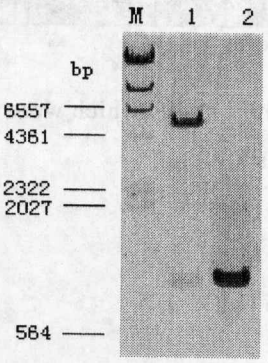

Fig. 3 Digestion identification of recombinant plasmid pFastBacHTb-*iap* using restriction endonuclease

M, DNA Marker;

1, pFastBacHTb-*iap*/ *Bam*H I+*Eco*R I;

2, PCR result of recombinant plasmid PMD18T- *iap*;

3.3. PCR identification of recombinant Bacmid-iap

Recombinant Bacmid-*iap* DNA as template, the pUC/M 13 primers were used for PCR amplification. Electrophoration result of PCR products showed that a specific band with 3.5 kbp appeared, to the negative control of 300 bp. It indicated that IAP gene occurred transposition in the bacteria DH5α.

3.4. Morphoulogic observation

The Bm cells infected with BmPAK6 virus changed

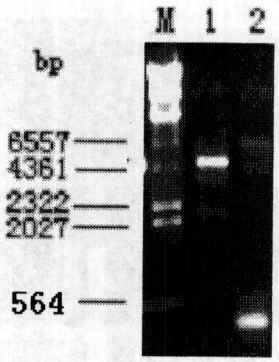

Fig. 4 PCR identification of recombinant Bacmid-*iap*

M, DNA Marker;

1 PCR products of Bacmid-*iap*;

2 PCR products of negative Bacmid

rounder with the nucleus expanded, and apoptotic bodies occurred. The Bm cells infected with r- IAP were normal in 24 h or 48 h. However, there were only mild symptoms of apoptosis in 72 h (Figure 4).The infected cells cultured in a medium containing X-gal, the medium was unchanged blue. This showed that the recombinant virus infected cells because LacZ had been damaged by virus gene inserted foreign genes. It can be inferred that the Bm IAP can be clearly delayed in the process of apoptosis.

3.5. RCR identification of recombinant virus iap

The silkworm cells infected with recombinant virus *iap* for 72 h were collected by proteinase K and RNase treatment as template for PCR. There was a fragment with the expected size of the *iap* gene (Figure 6).

3.6. ratio of cell survival after infection

To compare the survival of the cells, Bm cells were infected with recombinant virus *iap* and the virus BmPAK6, respectively. Survival of Bm cells was unconspicuous variation in the initial infection. However, Cell survival of Bm cells infected by BmPAK6 decreased obviously to those by r-*iap* ($P<0.05$). Meanwhile the change trend of the cells

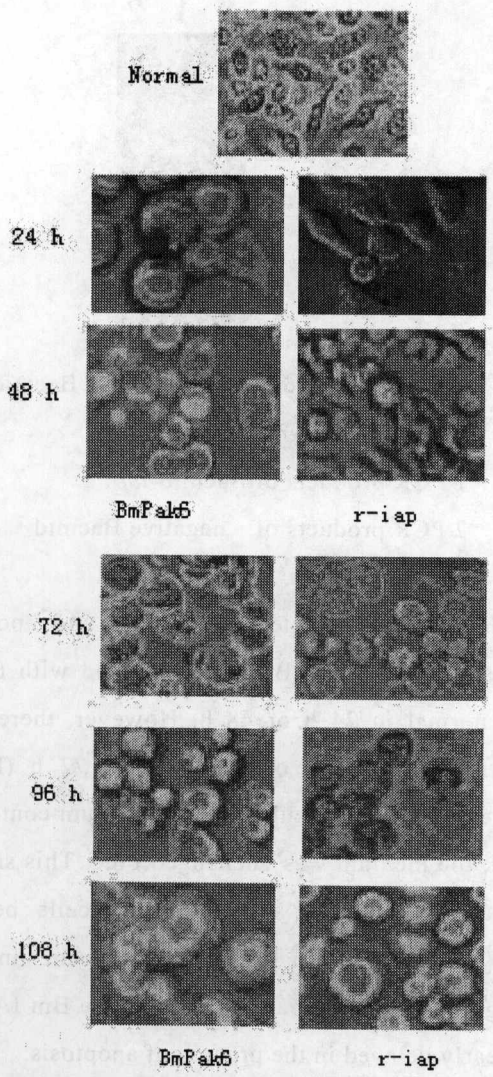

Fig. 5 *B. mori* cells infected by recombinant virus
Normal indicates normal silkworm cells;
BmPak6 indicates silkworm cells infected with BmPAK6 virus;
r-iap indicates silkworm cells infected with recombinant virus r-*iap*

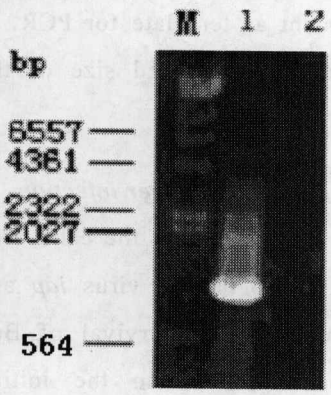

Fig. 6 RCR identification of recombinant virus *iap*
M, DNA Marker;
1, PCR products of r-*iap*;
2, PCR products of negative Bacmid

survival of Bm cells infected by MOI 10 recombinant virus *iap* was similar with it of the cells survival of Bm cells infected by MOI 1 recombinant virus *iap* ($P<0.05$). It can be inferred that the Bm *iap* could clearly inhibit cell apoptosis and extended life cycle of the virus.

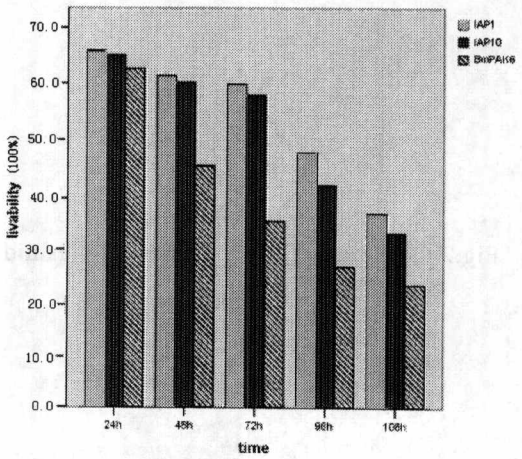

Fig. 7 the livability of cells which were infected using recombinant virus

1, Bm/r-iap (MOI=1);

2, Bm/r-iap (MOI=10);

3, BmPAK6 (MOI=10)

4. Conclusions

Cell morphology was observed at different times and cell survival rate was calculated. The experiments showed that the *iap* gene possessed anti-apoptotic role in the process of baculovirus infection. The over-expression of the *iap* gene at host cells could extend the life span and life cycle of virus.

Acknowledgements

This work was supported by the 973 National Basic Research Program of China (2005CB121005); National Natural Science Foundation of Jiangsu Education Communitte (02KJD18003).

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inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. J. Virol, 1994, (8): 2521-2525

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Expression Assay of *Bombyx Mori* Caspase-1 Gene in the Cells Treated with UV Irradiation and *Bm*NPV Infection

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Abstract: In order to investigate the role that Caspase-1 plays in apoptosis signaling pathway of *Bombyx mori* (Bm), we established a real-time fluorescent quantitative PCR to detect the expression of caspase-1 in the Bm cells with different treatment. Ultraviolet (UV) irradiation (20 mJ/cm2) and the infection of *Bombyx mori* nucleotide polyhedrosis virus (BmNPV) were applied to induce apoptosis in Bm cells, respectively. Bm cells were exposed in ultraviolet for 5 s, and the expression of caspase-1 reached its peak at 10 min after irradiation, which suggests that stimulation by ultraviolet increases the expression of caspase-1 gene in a short time. Inversely, viral infection reduced the expression of caspase-1 gene. Our results suggested that real-time fluorescent quantitative PCR used to detect the expression of caspase-1 is a reliable method and that Caspase-1 may be an upstream molecule in apoptosis pathway caused by UV irradiation and viral infection.

1. Introduction

Apoptosis is a normal physiological cell suicide program that is highly conserved among vertebrates and invertebrates [1-3]. Apoptosis, as a host defense, plays a critical role during normal development and tissue homeostasis, eliminating unwanted cells, including damaged and virus-infected cells, from the organism [4-6]. It has been demonstrated that the family of cysteine-dependent aspartate-specific proteases (Caspases) are essential components in the execution of apoptosis [7-11]. To date, while dozens of Caspases, which can be subdivided into three subfamilies based on their sequence similarities, have been identified in mammalian, only two caspase genes of *Bombyx mori*, a model insect, could be found in NCBI with their functions unknown. Apoptosis can be induced by many factors such as UV irradiation, viral infection, hormones, and high temperature. The aim of this study was to investigate the role of Caspase-1 in apoptosis signaling pathway. UV irradiation and viral infection were applied to induce apoptosis in Bm cells and real-time quantitative PCR was used to detect the changes in the expression of caspase-1.

2. Materials and Methods

2.1 Bm cells culture and treatment

The Bm cell line was cultured in TC-100 insect cell culture medium (Gibco, USA) supplemented with 10 % fetal bovine serum (FBS) at 27 °C. The wild-type (wt) BmNPV isolate was propagated in Bm cells. UV irradiation and viral infection were applied to treat Bm cells, respectively. The cells were plated at a density of 1–5 × 10^6 cells/mL in 24-well plates. The cells, with a very thin layer of PBS were irradiated for 5 s under UVA and UVB lamps. The total dosage irradiated to these cells, measured by an International Light Inc. (Newburyport, MA) radiometer fitted with a UV detector, was 20 mJ/cm^2. After irradiation, the cells were again cultured in TC-100 insect cell culture medium and were by turns collected after incubation of 0 h,

10 min, 30 min, 1 h, 4 h, 6 h, and 12 h. The Bm cells were infected with BmNPV at an MOI of 1.0 and the cells were collected respectively at 0 h, 4 h, 8 h, 12 h, 24 h, 36 h, 48 h after infection.

2.2 RNA extraction

Total RNA was extracted from the collected cells by use of Trizol (Invitrogen) according to the manufacturer's protocol. Contaminating genomic DNA was removed by Rnase-free Dnase I (Promega). The concentration of the RNA was assessed using GenspecIII(Hitachi Genetic systems), a spectro- photometer, and the integrity of the RNA was assessed by running 2 μl of RNA on a 1 % EB/agarose gel. And the RNA was stored at -70℃ until ready to use.

2.3 Reverse transcription

2 μg DNase-treated RNA was reverse- transcribed to single-stranded cDNA in a 20 μL reaction containing 0.2 μM oligo-dT, 0.5 mM of each dNTP, 5 μL M-MLV 5×reaction buffer and 200 U M-MLV reverse transcriptase (Promega). The thermal cycling profiles are as follows: 65 ℃ for 5 min, 37 ℃ for 60 min, 75 ℃ for 5 min. The resultant cDNA was stored at -20 ℃ until ready to use.

2.4 Primer design and real-time PCR

Primers used for the real time PCR amplification of caspase-1 and actin were selected based on the sequences available in GenBank. The primers were selected using PrimerSelect (DNAStar Inc., Madison, WI) and synthesized by Shenergy Biocolor Co (Shang-
hai, China).A forward primer (5'-ctgtggtattacttcactgc-3') and a reverse primer (5'-tttgagtaccatagtttct-3') were designed for the specific detection of caspase-1 in Bm (Genbank Accession Number: AF448494). Another pair of primers (Forward primer: 5'- GGATGTCCA CGTCG CACTTCA-3' and Reverse primer: 5'-GCGCGGCACT CGTTCACTACC-3') were designed for the specific detection of actinA3 in Bm (Genbank Accession Number: X04507) which was used as endogenous control in real time PCR.

Real-time PCR amplifications were performed to examine the relative expression of caspase-1 in treated Bm cells in the sequence detection system (Stratagene, USA). Duplicates of 0.5 μL cDNA from each reverse transcription reaction were used as real time PCR templates. The reactions were performed in a total volume of 50 μL using SYBR premix EXTaq^{TM} perfect Real time kit (TaKaRa, Dalian, China) as recommended by the manufacturer. The following MX3000P thermocycling program was used: denaturation program (3min at 95°C), amplification and quantification program repeated 40 times (10 s at 95 °C, 30 s at 58 °C and 20 s at 72 °C with a single fluorescence measure- ment), melting curve program (55 °C to 95 °C with a heating rate of 0.1 °C/s).

The comparative Ct ($2^{-\triangle\triangle Ct}$) method was used to calculate fold changes at relative gene expression level for caspase-1 in Bm cells with different treatment. All statistical analyses were conducted by using SPSS14.0 statistical software. One-way ANOVA with LSD post-hoc test was used to analyze data of more than two groups. *P values*$<$ 0.05 were considered to be significant.

3. Results

3.1 Changes in the expression of caspase-1 in Bm *cells with UV irradiation*

Real-time PCR was used to detect the expression of UV-induced caspase-1 with actin A3 as the endogenous control. After SPSS14.0 software dealt with the experimental data, the result was shown in Fig.1. From this figure, we found that the expression level of caspase-1 reached its peak at 10 min after UV irradiation, and began to decrease to basic level at 30 min after UV irradiation and arrived at the lowest at 4 h after UV irradiation.

3.2 Changes in the expression of caspase-1 in Bm cells with BmNPV infection

After BmNPV infected Bm cells, the expression of caspase-1 was decreased continuously. It was hard to detect the expression of the gene at 36 h post infection. This evidence indicated that expression of the host genes was blocked by viral infection (Fig. 2).

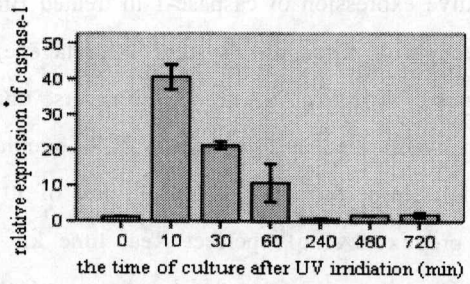

Fig.1 Significant fold difference (P< 0.05) in relative gene expression level of caspase-1 in *Bm* cells of different period culture after UV irradiation. The bars indicate the fold change of caspase-1 relative the endogenous control, actinA3 of *Bombyx mori*, (mean± S.E.M) in seven groups of cells. *Relative fold changes were calculated based on the formula: fold change $=2^{-\triangle\triangle Ct}$.

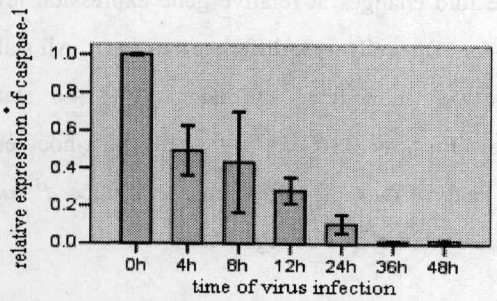

Fig.2 Significant fold difference (P< 0.05) in relative gene expression level of caspase-1 in Bm cells of different period culture after infection. The bars indicate the fold change of caspase-1 relative the endogenous control, actinA3 of *Bombyx mori*, (mean± S.E.M) in eight groups of cells. *Relative fold changes were calculated based on the formula: fold change $=2^{-\triangle\triangle Ct}$.

4. Discussion

Real time quantitative PCR has many advantages, such as accurate quantitative detection range, sensitivity and high precision in the detection of gene expression level and it is favored in the area of basic research and molecular diagnosis. At present, the main fluorescent modes include TaqMan probes and DNA-binding dye, such as SYBR Green I. Real time PCR with TaqMan probes has high specificity, but it is necessary to synthetic sequence-specific primers and fluorescent probes of high cost. SYBR Green I approach is practical and only sequence-specific primers are need to synthesize, but it is relatively poor in specificity and it is easy to be affected by the primer dimers. In this study, the reaction system is optimized and traps radiation and dissolution curves can be used to detect specificity.

It has been demonstrated that a caspase cascade plays an important role in the cell death process[12]. The induction of apoptosis combined with lots of different factors. The purpose of our study was to determine the role of Caspase-1 in apoptosis signaling pathway induced by different factors in Bm cells. It has been described that UV irradiation can induce cell apoptosis in present documents. UV irradiation may effect structural and functional modifications leading to immediate initiation of apoptosis followed by early membrane rupture. In this study, the expression level of caspase-1 reached its peak at 10 min after UV irradiation, and then declined to the basic level. This result suggested that UV leads to the early events in the apoptotic program and gave rise to the activation and expression of caspase-1 in the apoptotic pathway, resulting in cell death. Caspase-1 is an upstream molecule in UV-induced apoptosis pathway. Virus-induced apoptosis is associated with the activation of Caspases [13]. Furthermore, apoptosis acts as a host defense mechanism by which virally infected cells are eliminated to limit the propagation of viruses [14,15]. In this paper, our result indicated that virus infection caused reduced expression of caspase-1 gene. The change was the most obvious at 4 h after infection, and there were only small changes after this point. It suggested that Caspase-1 was an upstream molecule in virus induced apoptosis pathway. After infection, the virus expressed proteins as such P35 and IAP to block caspases expression and protein activation[15]. Furthermore, viral replication reduced host genes expression.

In conclusion, Caspase-1 may be an upstream molecule in apoptosis pathway caused by UV irradiation.

Acknowledgements

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