

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

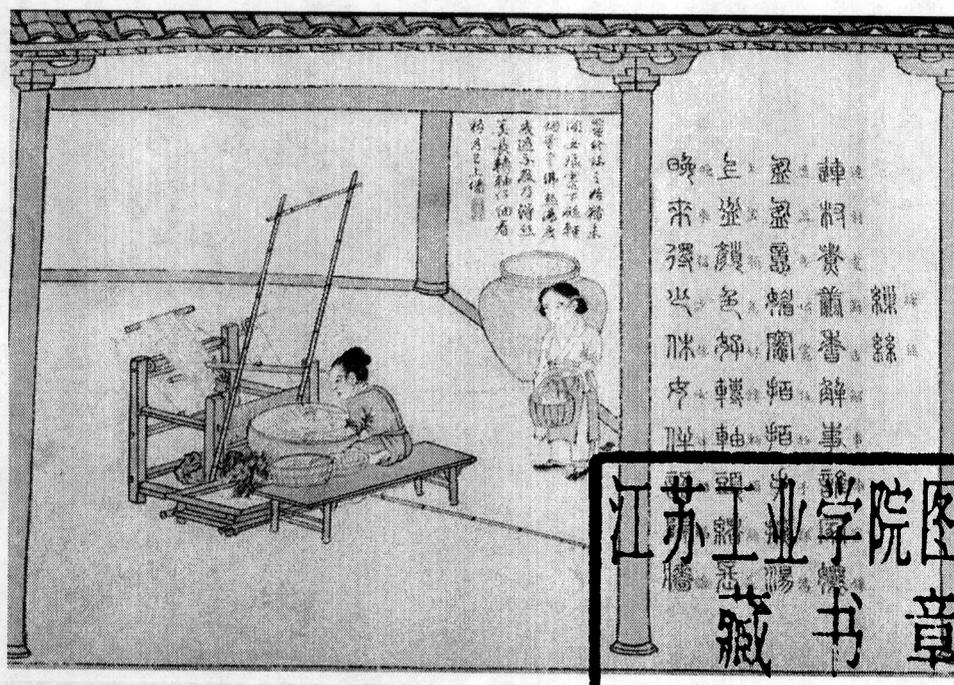
(I)

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

Suzhou 2007.9
Suzhou, Jiangsu, P. R. China

Sep. 13-14, 2007

Lun BAI



Sponsored by

China National Silk Coordinating Office

China National Silk Association

Jointly Organized by

Soochow University

The Chinese Society of Sericultural Science

The University of Manchester, UK,

North Carolina State University, USA,

Shinshu University, Japan,

The Hong Kong Polytechnic University



Chemical Industry Press

• Beijing •

图书在版编目 (CIP) 数据

现代丝绸、纺织、机电技术与进展=Researches and Progresses of Modern Technology on silk, Textile and Mechanicals: 英文/白伦, 芮延年主编. —北京: 化学工业出版社, 2007.8

ISBN 978-7-122-01157-2

I. 现… II. ①白…②芮… III. ①丝绸工业-国际学术会议-文集-英文②纺织工业-国际学术会议-文集-英文③机电工程-国际学术会议-文集-英文 IV. TS1-53; TH-53

中国版本图书馆 CIP 数据核字 (2007) 第 137165 号

责任编辑: 程树珍 金玉连

出版发行: 化学工业出版社 (北京市东城区青年湖南街 13 号 邮政编码 100011)

印 装: 化学工业出版社印刷厂

880mm×1230mm 1/16 印张 47 字数 1000 千字 2007 年 9 月北京第 1 版第 1 次印刷

购书咨询: 010-64518888 (传真: 010-64519686) 售后服务: 010-64518899

网 址: <http://www.cip.com.cn>

凡购买本书, 如有缺损质量问题, 本社销售中心负责调换。

定 价: 280.00 元 (上、下册)

版权所有 违者必究

Preface

The China International Silk Conference was held 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 held 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

I 桑蚕

1. Role of IAP Gene of <i>Bombyx mori</i> in the Process of Baculovirus Infection	1
2. Expression Assay of <i>Bombyx Mori</i> Caspase-1 Gene in the Cells Treated with UV Irradiation and <i>BmNPV</i> Infection	6
3. Study on Rapid Identification of Genus and Species Relationship of <i>Nosema Bombycis</i>	10
4. Studies on Rapid Identification the Classification of <i>Nosema Bombycis</i>	15
5. Development of Silk Industry in China	20
6. Genetic Diversity of Local Mulberry Varieties from Different Ecotypes Revealed by ISSRs in China	24
7. Study on Capsule of "ACV" of Anti-Virus Medicine for <i>Bombyx Mori</i>	29
8. Research on Polypoid and Apoptosis Induced by Heavy Metals in silkworm	34
9. Polymorphic Analysis of Silkworm Vitellogenins	39
10. The Database on the Origin of Silkworm Genetic Resources in Japan	42
11. TREE MULBERRY GERMPLASM AND UTILIZATION OF GENETIC RESOURCES	50
12. Differential Proteome Analysis of Salt Stress Responses in Mulberry by Two-dimensional Electrophoresis	58
13. Study on the High-throughput Analysis of Proteome in Silkworm, <i>Bombyx mori</i>	63

II 丝

14. Regression Analysis for Segment Size and Mean Size of Cocoon Filament	67
15. An Apparatus Monitoring Temperature and Humidity inside Rereeling Machine	71
16. Discussion about Digital Machine-Hour Capacity Design of Auto-Reeling	74
17. An Object Extraction Method Based on HSI Color Model to Determine the Proportion of Sericin in Raw Silk	79
18. Chemical Compositions and Morphologies of <i>Ornithoctonus Huwenna</i> Spider Dragline Silk	82
19. Analysis on the Mechanism of Water Infiltrating out of Cocoon during Cocoon Steaming	87
20. A Convenient Method to Prepare Slicing Samples of Raw Silk for Extracting the Cross-sectional Shapes	91
21. Design of Continuous Testing System of the Fineness of Bave	94
22. The Research of Measuring Interior and Exterior Temperatures of Cocoon in Cocoon Cooking Process	97
23. A Novelty Method to Measure the Diameter of Micro-aperture	100
24. The Study on Automatic Test System for Silk Cohesion	103
25. Study on the Grading of the Coefficient of Variation of the Raw Silk Size in the Electronic Testing	108
26. Study of Temperature and Humidity of Chuan Xi Continuous Cocoon Drying Machine	111
27. Antibacterial Performance of <i>B.mori</i> Silk Fabric Treated with Nano-ZnO	114
28. Structure and Performance of the Silver-Containing <i>B.mori</i> Silk Fiber	118
29. Structure and Performance of <i>B.mori</i> Silk Treated with Chitosan Nanoparticle	122
30. Outer Diameter Measurement of Raw Silk Thread by Using Laser-Scan Micro-Gauge System	126
31. Development of the Various Thick Silk Reeling Machine	129
32. Effects of a Ultrasonic and salt-shrunk Treatment on the Structure and Physical Properties of <i>Bombyx mori</i> Silk Fibroin Fiber	135
33. Polyvinyl Alcohol Nanofibers Containing Metallophthalocyanines	142
34. The Effect of Spinning Speed on the Structure and Properties of <i>Bombyx mori</i> Silkworm Silk	145

35. Statistical Analysis of Silkworm Spinning Behaviour under Abnormal Circumstances	151
36. Fabrication and Characterisation of Ultra-Fine Powders from Mulberry, Eri and Muga Silk Fibres	154
37. Eri Silk Fibre Attributes and Sliver Preparation	165

III 纺织绸

38. The Method and Application of Fabric Style Design based on the Style Evaluation of Fabric	174
39. Subjective and Objective Assessment and Analysis of the Handle of Fibriia-Style Fabric	179
40. Effect of guide plate gauge of a web cleaner installed on a carding machine on card sliver quality	186
41. Computer Aided Design for Fabric structure	189
42. Research on Influence of Thin Machine-sewing Fabric's Properties and Diagonal Way on Seam Pucker	193
43. Electrostatic Polymer Process of Polyolefin Fibrous Membrane	198
44. The Mechanical Properties of Nylon ATY for Technical Fabrics	201
45. The Mechanical Properties of PET ATY for Car-Seat Fabrics	204
46. An AHP-based Approach to the Optimum Selection of Fabrics Used for Close-fitting Garments	207
47. Group Decision Making of Fabric Hand and Its Intelligent Prediction	210
48. Analysis of Melt Flow in the Melt-blown Die Using Computational Fluid Dynamics	216
49. Preference of High-Performance Fabric Attributes through Conjoint Analysis	218
50. Investigation of the Perception of Fabric Hand Using Functional Magnetic Resonance Imaging	221
51. Objective Hand of High-Performance Silk Fabrics	225
52. A Study on Mechanical Properties of Nanofiber Nonwoven Fabric before/after Laminating Process	229
53. Effect of Amino Acid Treatment on Fabric Hand of Towel Cloth	231
54. Improving Functionality of Korean Paper (<i>Hanji</i>)	233
55. Study on Properties Changes of Laminated Nanofiber Nonwoven Fabric by Wash Fastness Testing	237
56. Research on the Automatic Detection of Fabric Thread Density	240

IV 染整

57. A Study of Inclusion Complex of Miconazole Nitrate with β -Cyclodextrin and Its Application on Protein Fabrics	243
58. The Dye Behavior of the Cationic Modified Silk	246
59. Dyeing Mechanism of Natural Plant Dye R. Coptidis on Silk	250
60. Preparation of End-H Fluoroacrylate Copolymer Emulsions and Application on Textile Finishing	254
61. Dyeing of Silk Fabric Using Rhubarb Extract	259
62. Dyeing of Tannin Treated Silk Fabrics with Alizarin Red S & Fe/Al Mordants	262

V 服装

63. Evaluation of the Fitting and Aesthetic Performance of Anti-Static and Non-Particle Clothing	267
64. The Clothing Pressure Measurement and Analysis of Woman's Suits	272
65. The Objective Evaluation Methods of Wrinkle Effect	276
66. The Optimization of Clothing Sewing Assembly Line	281
67. Study on Automatic Body Measurement Based on Apparel Application	285
68. Segmentation of Consumer Preference for Mountaineering Jacket Fabrics	288
69. An Empirical Analysis on Seam Quality of Cotton Woven Fabrics for Different Sewing Condition	291
70. Prediction of the Main Girths of Human Body Based on Photogrammetric Measurement	300
71. Silhouette Extract from Human Body Image Based on Color Clustering Analysis	303

72. Effect of Pressure Exerted on Ankle on the Skin Blood Flow of Lower Distal	306
73. Study on Preference of Female College Student toward Color and Style of Garments	309
74. Study on Pressure under Different Type Bras	312
75. Study on Photogrammetric Measurement of Human Body for Garment Design	315
76. Prediction on the Heat and Moisture Transfer Property of Multi-layers Garments	317
77. Application of PVDF Piezoelectric-film Sensor to Plantar Pressure Measurement	322
78. Study on Tactile Comfort of Spun Silk Fabrics	327

VI 材料

79. Study on Air Purification Capability and Applications of the Bamboo Charcoal Fibers	330
80. Fractal Characterization Analysis for Crepe Fabrics Surface Based on FTP	335
81. The Preparation of Silk Fibroin Microspheres	341
82. Preparation of Silk Fibroin Film Cross-linked by Carbodiimide (EDC)	346
83. Electrospinning of polyamide 6/66 copolymer nano-scale fiber yarns and their structure and mechanical properties	352
84. Preparation and Performance of Water Transmission Fabric without Finished by Hydrophilic Additives	358
85. Development and Performance Analyses of the Needle-punched Cocoon-outer-floss Nonwoven	362
86. Study on the Structure of Electrospun Fibers from Regenerated Samia Cynthia Ricini Silk Fibroin	366
87. Fourier Transform Infrared Spectrometric Analysis of Protein Secondary Structures of Regenerated Antheraea Pernyi Silk Fibroins	372
88. Mechanical Properties of Casein-acrylonitrile Graft Copolymer fiber	377
89. Study on the Property of the Electrospinning Silk Fibroin (SF)/gelatin Blend Nanofibers for Scaffolds	381
90. Structure and Properties of Ramie Fiber by Sodium Periodate Treatment	386
91. Study on the Oxidized Cotton Fiber Treated with Sericin	390
92. Structure and Properties of Wool Keratin Coated Cotton Fiber	395
93. Aggregate Structure of Cotton Fiber Treated with Sodium Periodate	399
94. Influence of Alkali Pretreatment on Selective Oxidation of Cotton Fiber	403

VII 其它

95. The source and development of folk home textile artistic design	407
96. Measurement and Evaluation of Erosion Characteristic for Fiber and Yarn	413
97. Improving Tear Characteristics of Vulcanized Rubber by Filling with Vapor-Grown Carbon Fiber	420
98. A Study of the Growth Strategy of Disposition Extension from E.LAND	425
99. Database of Asian Natural Fiber Resources	429
100. Imitating (Mimetic) and Value of Luxury Brand Pret-a-Porter	431
101. The Influence of Lifestyle on Clothing Consumption	437
102. Laser Electrospinning	442
103. A method to evaluate interfacial shear strength in a signal fiber composite	443
104. Clothing Comfort Evaluation of Women's Undergarment Made of Silk Spun Yarn	445
105. Influence of Shoulder Pattern on Kinetic Performance of Men's Suit Jacket	448
106. Research of Chinese Juvenile Body Measurement Method for Clothing	451
107. Cotton Fabrics on the Silk Road	456
108. Silk Trade between the Byzantine Empire and China during 6thC-10thC	459

109. Investigations of Collagen Absorption and Desorption Properties of Polyamide 6 / Polypropylene Flocking Nonwovens	462
110. Development and Research of the System Platform of Middle-Seniors' Costume Intellectualized Pattern-modifying Based on Body Shape	468
111. A Survey and Analysis of Girls Bra Wearing Condition	472
112. Dyeing Properties of <i>B.mori</i> Silk Fabric Finished with Nano-TiO ₂ and Chitosan	477
113. Gelation behaviour of a-PVA solutions in wide range of standing temperature and different DMSO/H ₂ O blending ratio	480
114. Structure and Properties of Silk Fibroin Grafted with Dimethylaminoethyl Methacrylate by ATRP in Water Aqueous	485
115. Embroidered and Woven <i>Tangka</i> as well as Chinese Textile Technology	489

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

Wu Hui-ling¹, LI Xi-bo¹, Li Bing², Shen Wei-de², Wang Wen-bing^{1*}

¹Institute of Life Sciences, Jiangsu University, Zhenjiang 212013, China;

²School of Life Sciences, Soochow University, Suzhou 215123, China;

Tel : 86-511-8791702 ; Fax,86-511-8791923 ;

E-mail : wenbingwang@ujs.edu.cn

Abstract: Inhibition of apoptosis protein (iap) factor was amplified 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 transferred 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- AGCGGATAACAATTTACACGGGA.

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).

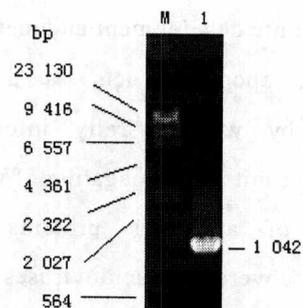


Fig. 1 The result of RT-PCR of *iap* gene from *B. mori*
M, DNA molecular weight marker;
1, PCR products of *iap* gene

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

The recombinant plasmid was identified by digestion of *EcoR* I and *BamH* 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).

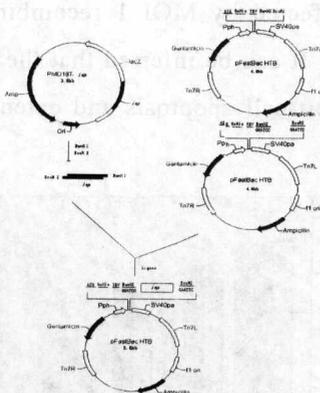


Fig.2 Consturction of recombinant plasmid pFastBacHTb-*iap*

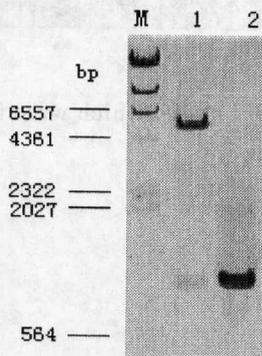


Fig. 3 Digestion identification of recombinant plasmid pFastBacHTb-*iap* using restriction endonuclease

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 DH5 α .

3.4. Morphoulogic observation

The Bm cells infected with BmPAK6 virus changed

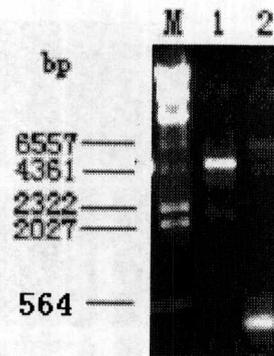


Fig. 4 PCR identification of recombinant Bacmid-*iap*

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 un conspicuous 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

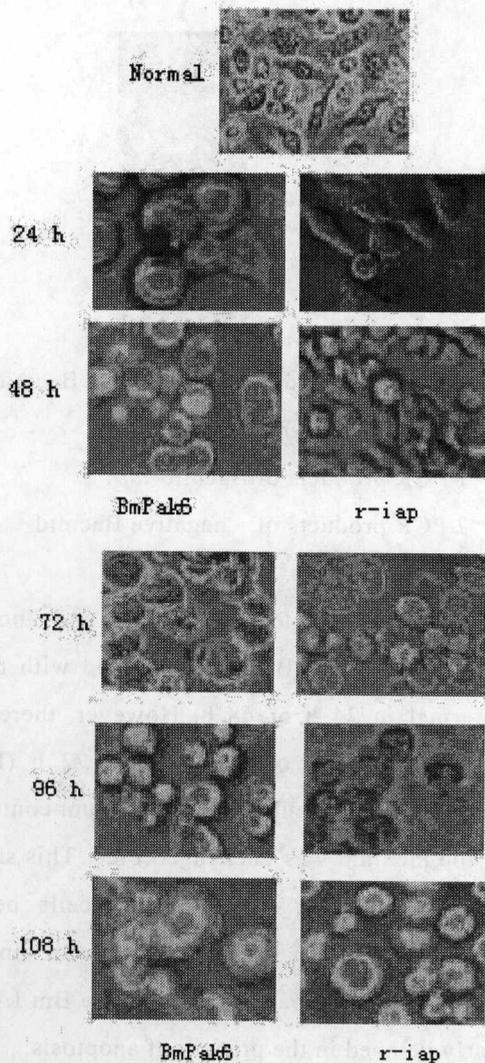


Fig. 5 *B. mori* cells infected by recombinant virus Normal indicates normal silkworm cells; BmPAk6 indicates silkworm cells infected with BmPAK6 virus; r-iap indicates silkworm cells infected with recombinant virus r-iap

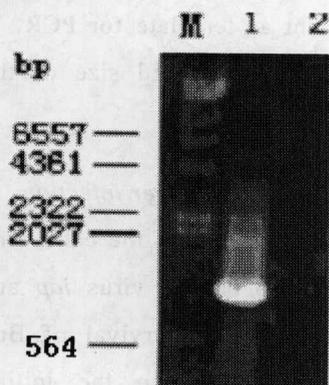


Fig. 6 RCR identification of recombinant virus *iap*
 M, DNA Marker;
 1, PCR products of r-iap;
 2, PCR products of negative Bacmid

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.

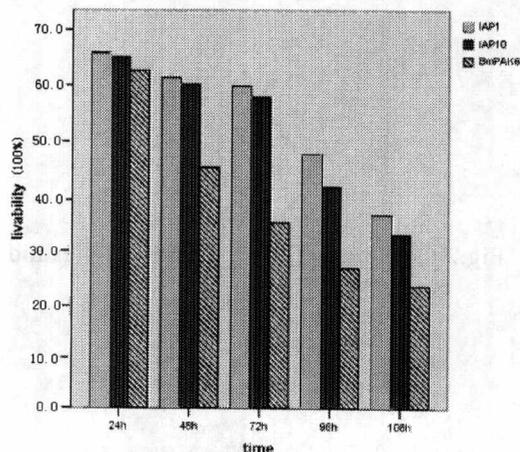


Fig. 7 the livability of cells which were infected using recombinant virus

- 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).

References

- [1] Birnbaum M, Clem R, Miller L. An apoptosis-

- inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. *J. Virol*, 1994, (8): 2521-2525
- [2] Clem RJ, Duckett, CS. The IAP genes: unique arbiters of cell death. *Trends Biochem. Sci.*, 1998, (23): 159-162
- [3] Hinds MG, Norton RS, Vaux DL, *et al.* Solution structure of a baculoviral inhibitor of apoptosis (IAP) repeat. *Nat. Struct. Biol.*, 1999, (6): 648-651
- [4] Sun C, Cai M, Gunasekera AH, *et al.* NMR structure and mutagenesis of the inhibitor-of-apoptosis protein XIAP. *Nature*, 1999, 441(4): 818-822
- [5] Vucic D, Kaiser WJ, Harvey AJ, *et al.* Inhibition of reaper-induced apoptosis by interaction with inhibitor of apoptosis proteins (IAPs). *Proc. Natl. Acad. Sci. USA*, 1997, 94 (9): 10183-10188
- [6] Vaux DL, Korsmeyer SJ. Cell death in development. *Cell*, 1999, (6): 245-254
- [7] Stellar H, Grether ME. Programmed cell death in *Drosophila*. *Neuron*, 1994, (13): 1269-1274

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

Wu Yan¹, Wang Hai-xia¹, Li Bing², Shen Wei-de², Wang Wen-bing^{1*}

¹ Institute of life sciences, Jiangsu University, Jiangsu Zhenjiang 212013, China;

² School of Life Sciences, Soochow University, Suzhou 215123, China

Tel: 0511-8790629

E-mail: wenbingwang@ujs.edu.cn

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 × 10⁶ 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/cm². 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 μ L of RNA on a 1 % EB/agarose gel. And the RNA was stored at -70°C until ready to use.

2.3 Reverse transcription

2 μ g DNase-treated RNA was reverse-transcribed to single-stranded cDNA in a 20 μ L reaction containing 0.2 μ M oligo-dT, 0.5 mM of each dNTP, 5 μ L M-MLV 5 \times reaction buffer and 200 U M-MLV reverse transcriptase (Promega). The thermal cycling profiles are as follows: 65 °C for 5 min, 37 °C for 60 min, 75 °C for 5 min. The resultant cDNA was stored at -20 °C 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 (DNASar Inc., Madison, WI) and synthesized by Shenergy Biocolor Co (Shanghai, China). A forward primer (5'-ctgtggtattactcactgc-3') and a reverse primer (5'-tttgagtaccatagttct-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 CGTTCCTACC-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 μ L cDNA from each reverse transcription reaction were used as real time PCR templates. The reactions were performed in a total volume of 50 μ L using SYBR premix EXTaq™ 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 measurement), melting curve program (55 °C to 95 °C with a heating rate of 0.1 °C/s).

The comparative Ct ($2^{-\Delta\Delta C_t}$) 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).

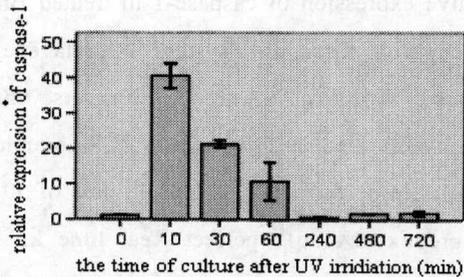


Fig.1 Significant fold difference ($P < 0.05$) in relative gene expression level of caspase-1 in *Bm* cells of different period culture after UV irradiation. The bars indicate the fold change of caspase-1 relative the endogenous control, actinA3 of *Bombyx mori*, (mean \pm S.E.M) in seven groups of cells.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

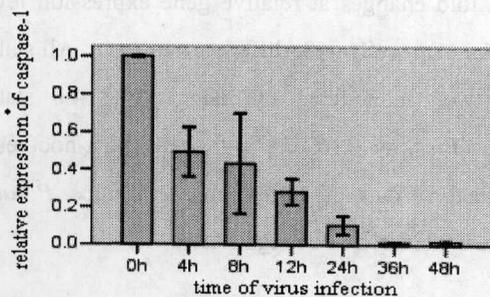


Fig.2 Significant fold difference ($P < 0.05$) in relative gene expression level of caspase-1 in *Bm* cells of different period culture after infection. The bars indicate the fold change of caspase-1 relative the endogenous control, actinA3 of *Bombyx mori*, (mean \pm S.E.M) in eight groups of cells.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

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

This work was supported by the 973 National Basic

Research Program of China (2005CB121005); National Natural Science Foundation of Jiangsu Education Communitte (02KJD18003) .

References

- [1] Jacobson MD, Weill M, Raff MC. Programmed cell death in animal development. *Cell*, 1997, 88:347-354.
- [2] Steller H. Mechanisms and genes of cellular suicide. *Science*, 1995, 267:1445-1449.
- [3] Kumar S. Caspase function in programmed cell death. *Cell Death Differ.*, 2007, 14(1):32-43.
- [4] Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*, 1972, 26(4): 239-257.
- [5] Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol.*, 1980, 68: 251-256.
- [6] Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science*, 1995, 267: 1456-1462.
- [7] Salvesen GS, Dixit VM. Caspases: intracellular signaling by proteolysis. *Cell*, 1997, 91: 443-446.
- [8] Cryns V, Yuan J. Proteases to die for. *Genes Dev*, 1998, 12: 1551-1571.
- [9] Thornberry NA, Lazebnik Y. Caspases: enemies within. *Science*, 1998, 281(5381): 1312-1316.
- [10] Earnshaw WC, Martins LM, Kaufmann SH. Mammalian caspases: structure, activation, substrates and functions during apoptosis. *Annu Rev Biochem*, 1999, 68: 383-424.
- [11] Chang HY, Yang X. Proteases for cell suicide: functions and regulation of caspases. *Microbiol. Mol Biol Rev*, 2000, 64: 821-846.
- [12] Liu Q, Chejanovsky N. Activation pathways and signal-mediated upregulation of the insect *Spodoptera frugiperda* caspase-1. *Apoptosis*, 2006, 11(4):487-496
- [13] Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J. Human ICE/CED-3 protease nomenclature. *Cell*, 1996, 87:171.
- [14] Clem RJ, Miller LK. Control of programmed cell death by the baculovirus genes p35 and IAP. *Mol Cell Biol*, 1994, 14: 5212-5222.
- [15] Saleh M. Caspase-1 builds a new barrier to infection. *Cell*, 2006, 126(6):1028-1030.