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Signal Transduction Pathway of the Protective Effects of Ginkgolides on Chemical Hypoxia Injury to Cortical Neurons

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Ginkgolides (Gin), mainly including A, B, C, J, M and belonging to terpene lactone are one of the main active constituents in the extract of leaves of *Ginkgo biloba* L. Gin B is a specific and potent antagonist of platelet-activating factor (PAF). Some animal and clinical data show the potential anti-ischemic/hypoxic effects and obvious protective effects of Gin on neurons, however the mechanisms of which and specially the signal transduction pathway of the protective effects of Gin are still unknown. Using primary cultured mouse cortical neurons treated with KCN (0.5 mM) induced hypoxia, we observed the effects of Gin (37.5 µg/ml) on the neuronal morphology and viability. The effects of Gin on the expression of immediate early gene (c-fos and c-jun) and MAPK signal transduction pathway induced by chemical hypoxia were assessed by Western blot. Main results are as follows. (1) Under phase-contrast microscope and transmission electromicroscope, many neurons treated by KCN were found shrunken with fragment neuritis, the disappearance of network, the condensed nuclear chromatin, the irregular nuclear membrane, the blebbing of cell membrane and the forming of apoptotic bodies. All these alteration in cell morphology could be attenuated by the pretreatment of Gin 24 h before being treated with KCN. (2) As determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay and the release of LDH (lactate dehydrogenase), the pretreatment of Gin for 24 h could increase the neuronal viability, enhance the cytomembrane stability and decrease the neuron death rate. (3) There was no expression of c-fos and only a little of c-jun in the control. The treatment of KCN for 2 h induced the expression of c-fos, which increased by the pretreatment of Gin for 24 h. However, the expression of c-jun enhanced obviously with the treatment of KCN for 2 h while the pretreatment of Gin for 24 h could decrease it to a very low level. (4) There is no difference of the expression of the total MEK and ERK kinase among the control, the KCN group and the Gin+KCN group. However, the expression of phosphorylated MEK (p-MEK) in the control was a little and increased when neurons exposed to KCN for 4 h., which increased further for the pretreatment of Gin for 24 h. There was only low expression of both p-ERK1 and p-ERK2 in the control, and only a bit of increase in the expression of p-ERK2 after the treatment of KCN and the pretreatment of Gin. Compared with the control, the levels of p-SAPK and p-JNK increased by the inducement of KCN and inhibited obviously with the pretreatment of Gin. In the cascades reaction of MAPKKK-MAPKK-MAPK, all the kinases would be activated when phosphorylated by the upper kinase. In general, the proliferation of cell increases if the ERK cascade is predominant, while the apoptosis starts if SAPK/JNK is predominant. As c-JUN is one of the substrate of SAPK/JNK, the activated SAPK/JNK could phosphorylate c-jun and then form transcription factor AP-1 and involve in the expression of apoptosis associated gene. Take together, Gin could decrease the expression of c-jun and p-SAPK and p-JNK induced by KCN. The protective effects of Gin on neurons against KCN-induced hypoxia may be associated with the inhibition of activity of SAPK/JNK.

Effects of Kidney—Tonifying Herbs on the Glucocorticoid Receptor (GR) mRNA Expression in Hippocampal Subfields of Aging Rats

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Objective To study the effect of Kidney—tonifying herbs (Zuogui Wan or Yougui Wan) on the glucocorticoid receptor (GR) mRNA expression in hippocampal subfields of aging rats. **Methods** Male SD rats were grouped as follows: (1) young control group; (2) old control group; (3) old group with Zuogui Wan; (4) old group with Yougui Wan. Using *in situ* hybridization, GR mRNA expression in subfields of the hippocampus was observed. **Results** Compared to young control group, the GR mRNA expression in hippocampal subfields of aging rats was weakened obviously. Meanwhile, the GR mRNA expression of the old group with Zuogui Wan and the old group with Yougui Wan was improved in DG of hippocampus. The GR mRNA expression of the old group with Zuogui Wan was improved obviously in CA1, while the effect of the old group with Yougui Wan was not clear. But in CA2 of hippocampus, compared to old control group, neither of the two groups was obvious. In CA3, the old group with Yougui Wan improved the expression obviously, while the effect of the old group with Zuogui Wan was not obviously the GR mRNA level of old rats weakened obviously. Meanwhile, the GR mRNA expression of the old group with Zuogui Wan and the old group with Yougui Wan were improved. **Conclusion** Aging maybe relate to the imbalance of hippocampus' regulating to the HPA axis; Zuogui Wan and Yougui Wan can retard aging through improving the function of hippocampus to the HPA axis.

The Expression of ppGalNAc-T2 in Different Tumor Cells and the Effect of Radiation

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Objective To study on the expressional difference of Polypeptide: N- Acetylgalactosaminyltransferase2 (ppGalNAc-T2) in several different tumor cells and to observe the effect of γ -irradiation to the expression of ppGalNAc-T2 in K562 Leukaemia cells. **Methods** The expression at mRNA level of ppGalNAc-T2 in six different tumor cells were examined by reverse transcription polymerase chain reaction (RT-PCR). Then, we used the method of *in situ* reverse transcription polymerase chain reaction (*in situ* RT-PCR) to verify the primary result in SGC-7901 gastric carcinoma cells. In observing the effect of γ -irradiation, K562 Leukaemia cells were respectively exposed to 5Gy, 10Gy, 15Gy γ -rays, after 12 hours, we tested the mRNA expression of ppGalNAc-T2 with the method of RT-PCR. **Results** The expression of ppGalNAc-T2 was different in six tumor cells. In SHG44 neuroglia cancer cells, the expression of ppGalNAc-T2 was extremely low, while in SGC-7901 gastric carcinoma cells and K562 Leukaemia cells, it's very high, the next was the ovary cancer cells (CD⁺), but in multiple medulla carcinoma cells, the expression of ppGalNAc-T2 was rare, the order was: (K562 Leukaemia cells)>(SGC-7901 gastric carcinoma cells)>[ovary cancer cells (CD⁺)>(multiple medulla carcinoma cells)>(NIH3T3 fibroblast cells and SHG44 neuroglia cancer cells); With the method of *in situ* RT-PCR, we found the expression of ppGalNAc-T2 was very high in cell line SGC-7901, and this result was accord with the above viewpoint; After exposed to γ -rays, the expression of ppGalNAc-T2 in K562 cells were somewhat reduced, and it was negatively relative to the dosage. **Conclusion** The expression of ppGalNAc-T2 has the particularity of cells and tissues, the level of the expression is comparative high in those cells which can excrete mucin glycoprotein, such as in SGC-7901 gastric carcinoma cells, and this result probably corresponds with the viewpoint that ppGalNAc-T2 is the key-enzyme in biosynthesis of O-glycan and O-glycan mostly consists in mucin; After the K562 cell lines were irradiated, the expression of ppGalNAc-T2 changed evidently, it hinted us that the structures and amounts of some adhesion molecules, fibronectins, collagens whose glycosylation was catalyzed at first by ppGalNAc-T2 probably changed, from this phenomena we primarily confirmed that ppGalNAc-T2 is relative to some biological specificity of cancer cells, such as tumour's invasiveness and migration. Grasping the varietal orderliness of this enzyme will help us to know the mechanism more in the course of tumor's generating and developing, this initial work is the foundation in studying the function of ppGalNAc-Ts and it will provide the experimental evidence to cancer diagnosis and clinical therapy.

Both the Core Region and Natively Unfolded Structure Are Required for the Aggregation of Some Neurodegenerative Disease-associated Proteins

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Amyloid-like aggregation or fibrillization of proteins is strongly associated with cellular dysfunctions, and some amyloidogenic proteins are relevant to neurodegenerative diseases, such as prion (PrP) to prion diseases, β -amyloid peptide (A β) to Alzheimer's disease and α -synuclein (α -Syn) to Parkinson's disease. Although sequence alignment showed little homology among these amyloidogenic proteins, we identified a consensus peptide, normally VGGAVVAGV (namely GAV motif), which may play vital roles in initiating protein aggregation or fibrillization [Du *et al.*, Biochem, 2003: 42(31)]. Structural analysis also revealed that the core sequences are often accommodated in the random coil regions, and the random coil structures are prone to transformation into β -sheet aggregates, which are toxic to neurons. Thus, we propose that aggregation of these neurodegenerative disease-associated proteins requires not only the core regions but also the natively unfolded structures. These studies may provide further insight into understanding of the molecular mechanism underlying the fibrillogenesis implicated in neurodegeneration as well as aid in drug design and development of transgenic models.

Cloning and Expression of Two $\Delta 6$ Desaturase Isozymes from *Mucor*

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γ -Linolenic acid (GLA, C18:3 Δ 6,9,12) is an essential polyunsaturated fatty acid for human. The biosynthesis of GLA in eukaryotes is accomplished by a desaturation of linoleic acid (LA, C18:2 Δ 9,12). This reaction is catalyzed by $\Delta 6$ fatty acid desaturase (D6d) through an insertion of a double bond between carbons 6 and 7 (the numbers counted from carboxyl-end) of the fatty acid substrate. D6d distributes in a wide range of organisms such as Zygomycetes and Oomycetes, and also some species in plants such as evening primrose and borage. Recently, genes coding for D6d were isolated from borage, nematode worm (*Caenorhabditis elegans*), fungus (*Mortierella alpina*), human, mouse and rat. In this paper, we described the isolation of two $\Delta 6$ desaturases from *Mucor circinelloides* and a differential response on their gene expression to the temperature changes.

Molecular cloning of two D6d To obtain the fragments of *M. circinelloides* D6d genes, two generate primers corresponding to the amino acid conservative sequences from the reported desaturases were synthesized. The RT-PCR and 5'-RACE were conducted with the total RNA as a template. The isolated mcD6-1 cDNA was 1404 bp in length and capable of coding for an open reading frame of 467 amino acids. Its deduced amino acid sequence showed 45.2% and 25.9% homology against $\Delta 6$ desaturase from *Mortierella alpina* and borage, respectively. On the other hand, the mcD6-2 cDNA isolated as a homolog of *Mucor rouxii* $\Delta 6$ desaturase was 1572 bp in length and could encode an open reading frame of 523 amino acids. The two enzymes shared three histidine clusters, two hydrophobic regions and a heme-binding HPGG motif found in cytochrome b5. Only mcD6-2 sequence contained a characteristic long insertion of unknown function.

Heterologous expression To examine the enzymatic functions of mcD6-1 and mcD6-2, the genes were inducibly expressed in yeast *S. cerevisiae*, in the presence of linoleic acid as a substrate. GC-MS analysis indicated the two desaturases could be expressed in the yeast.

Response of gene expression to low temperature To compare the expression levels of the $\Delta 6$ desaturase genes in *M. circinelloides* myceria grown at the different temperatures, the Northern blotting analysis was performed with the mcD6-1/mcD6-2 RNA probe at a

concentration of which it hybridized linearly with dilutions of the target genes. In the total RNA from the cells grown at 28 °C for 24 h, the amount of mcD6-2 transcripts was less than a half of mcD6-1 level. In contrast, the cultivation of cells at 15 °C for 24 h increased the amount of mcD6-2 transcripts to a level equivalent to the amount of mcD6-1 transcripts.

A Novel Gene Specifically Expressed during Pollen Development from Wheat with Multiple Polyadenylation Sites

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A novel pollen-specific full-length cDNA clone *TaPSG076* was isolated using suppression subtractive hybridization and 5'/3' RACE techniques. *TaPSG076* was shown by sequencing the 3' ends of the cDNAs to exhibit multi site polyadenylation. At least five different length transcripts produced from the single gene based on different poly(A) tail attachment sites. However, polyadenylation consensus sequence AAUAAA is not seen at the 3'-untranslated sequence. One of *TaPSG076* clone is 1335-bp long and contains a 299-bp 5' untranslated region, an open reading frame of 663 coding 221 aa with pI of 4.31. A Blast search reveal that this sequence did not show a significant similarity to any genes deposited the public database. Southern blot indicated that *TaPSG076* was a single copy. Northern blot and RT-PCR analysis indicated *TaPSG076* transcripts showed specific expression of transcript in mature pollen, and weak or undetectable signals in uninucleate microspore, immature seed, stem, young leave, root and ovary. Further analysis of the gametophytic expression pattern showed *TaPSG076* transcripts were undetectable in uninucleate and binucleate microspore and pollen at early stage, and were first detectable and increase rapidly at middle and late stages of pollen development with maximum levels at mature pollen and also expressed at high level in germinating pollen *in vivo*, suggesting that *TaPSG076* might play a role in pollen germination and pollen tube growth in addition to its function in maturation.

Study on Attenuation Characteristics of Biocontrol Strain Anti-8098A, *Bacillus cereus*, against *Ralstonia solanacearum*

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The present study dealt with the attenuation characteristics of bacterial-wilt-disease biocontrol strain Anti-8098A, *Bacillus cereus*, against pathogen *Ralstonia solanacearum* (RS). In order to distinguish the pathogenicity of RS, the attenuation index (radius of the center red ring/radius of the whole mycelium ring, on TTC culture medium) was established (Hayward, 1976), companying with the mortality of tomato tissue culture plantlets infested with the pathogeny. The RS virulent strain was recognized with an index less than 0.60, the RS avirulent strain with that greater than 0.80. Using Anti-8980A to treat virulent RS, the RS virulent strain with an index of less then 0.60 changed into avirulent one with an index of greater than 0.80 within 24 h treatment time. It was the first time to define the attenuation characteristic of biocontrol strain against RS.

In order to distinguish the attenuation effect caused by the biocontrol strain from other factors, such as cultural activity, physical treatment, chemical treatment and other bacteria etc., a series of experiments have been done. The results showed that the culture activity could not make the same strain of RS attenuated within 120 h in 10 generations. After treated with different times of physical factor ultrasonic, the RS virulent strains preserved the similar attenuation indexes and infection mortalities of tomato plants. Acting as a chemical factor, the streptomycin could inhibit the RS virulent strain, but couldn't attenuate it. 15 bacterium strains were used in the test of attenuating the RS virulent strain, only two stains of *Bacillus cereus* Anti-8098A and *Bacillus sphaericus* showed the attenuation activities, changing the RS virulent strain into the RS avirulent strain within 24 h. All of these results proved that the attenuation activity caused by the biocontrol strain was a new characteristic, differing from that caused by other factors.

In order to identify characteristics of the tested RS strain before and after attenuation of biocontrol strain Anti-8098A, the techniques of DNA-PCR, UV spectrophotometer, HPLC and protein electrophoresis were introduced. The results showed that the RS strain before treatment displayed no band for STRR primer in PCR analysis, while that of after treatment consisted of 6 bands. In the detection of UV photo-absorptions, the RS strain before treatment got a value of 0.640 at 450 nm wavelength, which significantly differing from that of after treatment with a value of 0.2644. Using HPLC detection, it showed significant differences between the RS strains before and after treatment. The protein electrophoresis results also revealed significant difference between the RS virulent strain before treatment and the RS avirulent strain after treatment. It was concluded that the biocontrol strain Anti-8098A had the attenuation characteristics to turn RS virulent strain into the avirulent one in 24 h.

Based on the attenuation theory, the experiments on control efficiency of biocontrol strain Anti-8089A were conducted on tomato plants in greenhouse. The tomato plants were infested with the 10¹⁰ CFU virulent RS strain firstly, and then treated with the biocontrol strain Anti-8089A fermented liquid with a concentration of 2×10¹⁰ CFU, while the water treatment was used as a control. The results showed that all of the RS strains isolated from tomato plants treated with Anti-8098A were RS avirulent stain, the control efficiency of Anti-8098A was 96% and the mortality of control was 98%. The similar results have also got from the field trial. It proved that the attenuation activity was one of the important mechanisms, when using the biocontrol strain Anti-8098A to control the bacterial wilt disease in tomato plants.

Purification and Identification of the Major Allergens in *Artemisia apiacea* Pollen

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Background Inhalation of pollen in the atmosphere provokes IgE-mediated responses of hay fever and allergic asthma in about 20% of

humans. *Artemisia* is the dominant source of allergens in china because of its prodigious production of airborne pollen. There are many species of *Artemisia* around the world, but *Artemisia apiacea* is most important. Purification and identification of major allergens of them have not been reported so far. **Objective** To identify and purify the major allergens from the *A. apiacea* pollen crude extracts. **Methods** *A. apiacea* was extracted in the coca's solution after being defatted. Proteins were then concentrated by means of saturated ammonium sulfate homogenous precipitation. Western blot was done to identify allergens using sera from subjects allergic to *Artemisia* pollen. DEAE-Cellulose DE-32 ion Exchange chromatography (IEC) was used for primary purification. The major allergen was further purified by Sephadex G-75 size exclusion chromatography and analysed by ESI-MS/MS. **Results** The SDS-polyacrylamide gel electrophoresis (SDS-PAGE) results showed that the crude extract of *A. apiacea* pollen contained at least 26 bands with the molecular weight ranging from 14–100 kD; there were seven main protein bands with the molecular weight being 63, 51, 43, 38, 35, 32, 27 kD respectively. The 51, 43, and 38 kD protein bands were identified as the major allergens by means of Western-blotting. The major and minor allergen of *A. apiacea* pollen were partially purified by DE-32 Ion Exchange chromatography (IEC). And the 43 kD major allergen was further purified by Sephadex G-75 size exclusion chromatography, whose bioactivity was detected by Western-Blotting. **Conclusion** This study has laid a theoretical base for the diagnosis and immunotherapy of allergic diseases.

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Construction and Primary Characterization of cDNA Expression Library of *Artemisia apiacea* Pollen

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Background Allergic diseases including extrinsic asthma (allergic asthma), allergic rhinitis etc., are defined as a worldwide important health problem by WHO. *Artemisia apiacea* pollen is one of the most important and common airborne allergens in China. *Artemisia* is difficult to standardize and contains a large number of non-specific proteins, therefore, characterization of *Artemisia*-derived allergens at the molecular level is an important step for the development of effective diagnostic and therapeutic approaches. **Objectives** (1) To construct the cDNA library of *A. apiacea* by molecular biological technique so as to provide the scientific basis for screening candidate gene of recombinant allergen of *A. apiacea* pollen. (2) To screen immunologically the cDNA library with *A. apiacea* allergic patients' serum, followed by isolating, identifying the specific recombinant allergen genes and having the genes sequenced, so as to provide the scientific basis for screening candidate gene of recombinant allergen of *A. apiacea* pollen. **Methods** Proteins were removed by using phenol: chloroform: isoamylalcohol solution. Total RNA was precipitated by isopropanol. mRNA was purified by Biotin-Oligo (dT). cDNA's first strand synthesis was catalyzed by moloney murine leukemia virus (MMLV) reverse transcriptase with NotI dT18 as primer. After addition of EcoRI adaptors, phosphorylation and NotI restriction enzyme digestion, the cDNA with 5'-EcoRI end and 3'-NotI end was directionally ligated with the EcoRI-NotI arms of the Lambda ExCell NotI/EcoRI/CIP vector, followed by Lambda DNA packaging and transfecting host cell *E. coli* NM522. According to phage plaques' clear/blue selection in the presence of IPTG and X-Gal, the cloning efficiency and recombinant quantity was calculated. cDNA library was then immunoscreened. **Results** The total number of recombinants obtained was 5.6×10^5 as measured by formation of plaque-forming unit (pfu), the proportion of recombinance is 100%, and the average length of inserted cDNA are about 1.1 kb. cDNA library were immunoscreened using the sera specific IgE of 30 patients allergic to *Artemisia* pollen. Three positive clones were identified and sequenced. **Conclusion** A lambda ExCell cDNA library was constructed from *A. apiacea* successfully and three positive clones were isolated, which laid a solid base for the further research.

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Study on the Anti-Tumor Mechanism of Podophyllotoxin and Its Derivatives

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Podophyllotoxin (PPT) is a well known natural product with a long history of use in folk medicine following its isolation, its derivative Etoposide (VP-16) has been successfully semi-synthesized and been proved to be a high activity anti-tumor medicine by clinic practices. We have used the pulse radiolysis technology to found that an electron adds to the aromatic ring on C-1 of PPT and its derivatives to produce an anion radical, which is then protonated to yield a neutral radical. A hydrogen radical adds to the lactone ring and a hydroxyl radical abstracts hydrogen from the C-4 hydroxyl group to produce a hydrogen abstraction radical. The reaction mechanisms of them are suggested. The rate constants for the formation and decay of the transient species have been determined. The oxidizing mechanism of VP-16 was studied by sodium persulfate, the reaction rate constant is determined as $4.04 \times 10^9 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$. The electron-transfer between VP-16 with tyrosine and VP-16 with GMP (guanosine-5'-monophosphoric acid) in aqueous solution are observed for the first time and the reaction rate constant were determined as $1.1 \times 10^8 \text{ dm}^3 \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$ and $3.16 \times 10^7 \text{ M}^{-1} \cdot \text{s}^{-1}$ respectively. These results may be useful to explain the anti-tumor mechanism of PPT and its derivatives.

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Inhibition of Androgen Responsive Element Decoy Oligonucleotide on the Promoter of Prostate Specific Antigen Gene

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Transcription factor decoy oligonucleotides have potential therapeutic application. The androgen receptor (AR) is a member of the steroid

receptor superfamily, namely, ligand-dependent transcriptional factor, which can bind to the androgen-responsive element (ARE) in the promoter region of prostate-specific antigen gene (PSA) and transactivate the gene transcription. To investigate the effect of exogenous androgen responsive element decoy oligonucleotide on the promoter of PSA gene. pGL3-PSA luciferase expression vector containing 640 bp DNA in the 5'-promoter region of PSA gene was constructed. Meanwhile, a 23-mer phosphorothioated ARE decoy oligonucleotide based on the deduced ARE sequence at the promoter region of PSA gene was synthesized. pGL3-PSA plasmid and ARE decoy were cotransfected into prostate cancer cell PC3-M by LipofectAmine™ 2000. Through detecting the activity of luciferase, the effect of ARE decoy on the promoter of PSA gene was studied. The results showed that activity of luciferase was significantly reduced in the ARE decoy-transfected cells, the promoter activity could be inhibited by 90%, though not in the cells transfected with the control oligonucleotide. And there were not significant differences of luciferase activities in different time after the PC-3-M cells transfected with ARE decoy oligonucleotide. ARE decoy oligonucleotide could be specifically bound by AR, that was determined by electrophoresis mobility shift assay (EMSA). And ARE decoy could also induce apoptosis of prostate cancer cell LNCaP, which was resulted from observation of the change in cell form, MTT assay, apoptosis DNA fragmentation assay and flow cytometry analysis.

Functional Analysis of Promoter Activity of *Bombyx mori* Nucleopolyhedrovirus Ecdysteroid UDP-glucosyltransferase Gene

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The different length respect to the upstream of translating initiation site ATG of ecdysteroid UDP-glucosyltransferase (*egt*) gene were amplified from *Bombyx mori* Nucleopolyhedrovirus (BmNPV) genome DNA of Zj-8 strain by the method of PCR. And the ATG was mutated to ATT. After the construction, the reporting plasmids pBmegt544-luc, pBmegt311-luc and pBmegt161-luc with the luciferase gene driven by above single *egt* promoter fragment were used to transfect insect cell strains and silkworm larvae of 5-instar mediated by lipofectin reagent for transient expression of luciferase. The results of the experiments showed that BmNPV *egt* promoter is not an early gene promoter. The expression product of luciferase gene driven by the *egt* promoter did not be detected until 18 h post transfection and its transcription activity required the transactivation of viral factors. BmNPV homologous region 3 (hr3), as an enhancer, increased the *egt* activity by over 1000 folds. While the exogenous insect moulting hormone (MH) and Juvenile hormone (JH) presented the dose-dependent effects on the *egt* promoter activity. The comparison on activity of different *egt* promoter fragment length indicated that 161 bp nucleotide sequence, upstream the translating initiation site ATG, can initiate the transcription of luciferase gene, though the activity was low. It suggests that the 161 bp sequence contains the basal elements of *egt* promoter. In contrast, the expression of luciferase gene driven by 311 bp and 544 bp nucleotide sequence of *egt* promoter fragment, respectively, presented similar expression but much higher than that of 161 bp. Thus we deduced that there are *cis*-regulatory elements for binding of viral transactivation factors. Meanwhile, the transfection and expression of above reporting constructions in silkworm larvae of 5-instar also need the transactivation of viral factors, demonstrating that BmNPV *egt* promoter is not an early but late gene promoter.

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Functional Analysis on the Promoter Activity of the Heat-shock-cognate 70-4 Gene from *Bombyx mori*

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The promoter region of the heat-shock-cognate 70-4 gene from *Bombyx mori* (*BmHSC70-4*), about 1.4 kb, was amplified from genomic DNA from the silkworm variety of Suju×Minghu by PCR method and cloned. By use of the plasmid with a reporter luciferase gene (*luc*) driven by *BmHSC70-4* promoter to transfect insect cells and 5th instar silkworm larvae, transient expression assays were carried out to investigate the effects of heat shock treatment, insect ecdysone (MH) and juvenile hormone analogue (JHA) on the *BmHSC70-4* promoter activity. The results demonstrated that the *BmHSC70-4* promoter activity was high in the normal conditions in BmN cells, sf9 cells and 5th instar silkworm larvae. By heat-shock treatment in 37 °C condition for 2 h, compared with the control, the *BmHSC70-4* promoter activity was increased by 4.40, 361.40, 5.44 fold in BmN cells, sf9 cells and 5th instar silkworm larvae, respectively. MH treatment had no significant effect, while JHA treatments showed typical dose-dependent effects on the *BmHSC70-4* promoter activity in BmN cells. JHA lower than 1.5 µg per ml in the media increased the *BmHSC70-4* promoter activity significantly, and 2–3 µg showed no significant effect, while 3–7 µg presented a negative significant effect on the *BmHSC70-4* promoter activity. The 50, 100 mg/kg JHA treatment with 5th instar silkworm larvae showed no significant effects on the *BmHSC70-4* promoter activity, while 150 mg/kg had a positive significant effect. From the above results obtained from *in vitro* and *in vivo*, we can deduce that the characteristic of *BmHSC70-4* promoter is both constitutive and inducible promoter.

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