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Heat shock response and mammal adaptation to high elevation (hypoxia)

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Abstract The mammal's high elevation (hypoxia) adaptation was studied by using the immunological and the molecular biological methods to understand the significance of Hsp (hypoxia) adaptation in the organic high elevation, through the mammal heat shock response. (1) From high elevation to low elevation (natural hypoxia): Western blot and conventional RT-PCR and real-time fluorescence quota PCR were adopted. Expression difference of heat shock protein of 70 (Hsp70) and natural expression of brain tissue of *Hsp70* gene was determined in the cardiac muscle tissue among the different elevation mammals (yak). (2)From low elevation to high elevation (hypoxia induction): The mammals (domestic rabbits) from the low elevation were sent directly to the areas with different high elevations like 2300, 3300 and 5000 m above sea level to be raised for a period of 3 weeks before being slaughtered and the genetic inductive expression of the brain tissue of *Hsp70* was determined with RT-PCR. The result indicated that all of the mammals at different elevations possessed their heat shock response gene. Hsp70 of the high elevation mammal rose abruptly under stress and might be induced to come into being by high elevation (hypoxia). The speedy synthesis of Hsp70 in the process of heat shock response is suitable to maintain the cells' normal physiological functions under stress. The Hsp70 has its threshold value. The altitude of 5000 m above sea level is the best condition for the heat shock response, and it starts to reduce when the altitude is over 6000 m above sea level. The Hsp70 production quantity and the cell hypoxia bearing capacity have their direct ratio.

Keywords: heat shock reaction, heat shock protein 70 (Hsp70), Western blot, RT-PCR, fluorescence quota PCR, high elevation hypoxia, mammal, stress.

1 Introduction

Biological environment adaptation refers to the phenomenon in which the biological shape structure and function adapt to a certain environmental condition for their living. In the perpetual flow of evolutional history, the organism has gained its capabilities such as behavior adaptation, shape change, reproduction adjustment and cellular effect to resist threats arising from the changing environment. The most common capability involves the rapid synthetic cell response to threats of the heat shock protein (Hsp) [1]. Internationally, the research on tracing biological evolution path through the molecular comparison has made an enormous breakthrough and the research on the heat shock protein in molecular evolution is mainly focused on this aspect.

The heat shock response is one kind of new biological forms that adapts to the adverse circumstance. It has become a hot research area since the heat shock response was discovered when Ritossa[2] studied the fruit fly salivary gland chromosome in 1962 and Tissieres[3] studied the fruit fly young insects in 1974.

The heat shock response, the response taken by cells after they are confronted with exterior stress, exists in the entire biosphere from bacterium to human beings. It is induced not only by heat stimulation[4], but also by some physical factors, such as ultraviolet ray, the beam, chemical factors, such as heavy metal, cell toxic agent, and the machinery stimulation, such as the hypoxia, coldness and malnutrition[5]. A special mention should be made to Hsp70 (hypoxia) since it has important cellular functions like resistance, and molecular companion. When Hsp70 is under stress at high elevation (hypoxia), it could strengthen the duration degree of the cells to the next possible harmful damage, maintain cells' normal physiological function metabolism and enhance the cell survival rate[6−9].

The heat shock response caused by all kinds of stress factors is called stress response (SR), and the protein thus produced is called heat shock protein, which could prevent organism or cells from being harmed to a minimum degree[10] .

The heat shock protein can produce a series of corresponding physiological reactions to slow down the cell damage and these functions are as follows:

The protein produces precipitation when it receives physical or chemical stimulations.

As the molecular companion, the heat shock response assists the protein in transporting among the cell organs.

It assists the newborn peptide chain to fold and prevent it from making any mistakes when folding[11−15].

As the DNA hydrolytic enzyme in the alkali base match, it assists in repairing DNA structure[14]. In addition, the Hsp, including the molecular companion, is a universal prevailing characteristic, namely, it is the denatured cell for the other proteins to reduce their pressures.

The Hsp has received great attention and got experimental stress in the organism, and its functions at molecular and the cell level have been understood.

A focal point emerges from the model and from the natural organism for Hsp to accept stress. The research on the whole eukaryocyte physiology, tissue and the organ in the Hsp includes ecological change and *Hsp* gene evolution. This focal point disclosed that (a) the Hsp expression possibly occurs in the nature; (b) all types of species have the *Hsp* gene, but their changes have their specific expression forms; (c) Hsp expression possibly has its connection with the resistant stress; (d) the Hsp expression types have their connection with Stress level to be received accordingly. But so far not a single answer could tell us the diversified *Hsp* gene, two mechanisms of the Hsp mediation and the endurance in organic level and evolution mechanism[16]. The present researches think that the heat shock protein may play a role in the species evolution through working as the promotion factor or "the sudden change vessel"[17−19].

Lerman[20] and the other researchers confirmed through their experiments that when environment changes the Hsp genetic material may have the quantitative change, and this kind of change can have the difference in the population scope and the natural selection may produce some functions to it. Rutherford[17] and his colleagues thought that Hsp may slow down the species phenotypic change. Cushion, namely, the natural selection is capable of increasing the heredity multiplicity of species and under the Hsp adjustment, the organism phenotypic change generally does not show up immediately until the species environment is

in danger.

Correspondingly, when the organism is in danger, Hsp is involved in some kind of mechanism to make the natural break into a selective change and this adaptation meets the selection demand. In other words, Hsp could affect the genetic expression under the selection pressure and correctly fold Hsp through adjusting the suddenly changed protein, or slow down the changing phenotypic protein through indirectly adjusting the signal extension to prevent the shape gene unfavorable to environment from expressing immediately so that the hidden gene favorable to environment could be expressed.

Christne Queitsch and some other researchers have further supported this view through their experiments. The national research on Hsp has progressed to the genetic level with a better understanding of the expression of *Hsp* gene and heat shock protein. However, the research has been limited to the fields like pharmacy, sports medicine, livestock veterinary, agriculture, etc. and the research on the ecological field has just started. But some foreign countries have started to study the ecological adaptation with Hsp expression many years ago. For example, Israeli scientists, Omer Chorsh, et al. have studied the adaptation capability of green sea anemone to the sea temperature changes along Mediterranean Sea Israel coast[21].Valeria Ramaglia, a Canadian scientist has studied the adaptation of western drawing turtle to the hypoxia through using Hsp70[22].

This paper is the first one in China to study the mammal's adaptation to the high elevation (hypoxia) through first using of Hsp70. And its true meaning lies in the situation that when mammals (human beings) go up to the high elevation area all of a sudden, they would have a series of symptoms like having difficulty in breathing, etc., or even die because they are not adapted to the high elevation. However, if they take a rest for a few days in a second high elevation area, they would overcome all these symptoms like having difficulty in breathing without apparent ill adaptation. Our research result has indicated that organism could be adapted to the high elevation (hypoxia), but not allowed to make it happen all of a sudden, it can only be allowed to go up gradually to make the organism have sufficient time to correspond to the stress adaptation[23]. The stress adaptation mechanism is a response to the heat shock and the heat shock protein production.

Hsp70 has become an essential contribution to the ecological environment and the contributions include the following:

(1) All the organisms have Hsp70 expression, and the different organism changes occur in their reaction time.

(2) The most Hsp70 expressions can return back to their normal physiological conditions.

(3) The Hsp70 is applied in the environmental monitoring as a biological marker[24].

(4) The heat shock response clearly enhances the biological survival and the endurance of organism[25] .

5) Hsp70mRNA expression is high in the biology with adaptation to circumstances or environment, the heat shock response is adjusted to adapt to the organism[26].

2 Materials and method

2.1 Reagent

The Hps70 standard molecular weight was protein produced by Serva Corporation. First antibody was mouse-anti-person Hsc70/Hsp70 monoclonal antibody produced by Beijing Zhongshan Company. Second antibody was sheep-anti-mouse IgG with the badge of HRP produced by Beijing Zhongshan Company.

DAB Coloration indicates di-aminobenzidines produced by Beijing Zhongshan Company. Trizol Reagent Box for Extracting RNA was produced by DingGu Company. M-MLV RT (100 u/μL), Rnasin and 5 X buffer was produced by Japanese TOYOBO Biology Company. DTT (100 mmol/L), and random primer of hexamer (20 μmol/L) were produced by DingGu Company. DNTPs(10 mmol/L. pH 7.5) was produced by Genview. Primer: The primer of the *Hsp* gene and the β-actin gene was synthesized by Beijing AuGCT Biology Company.

Instrument: PCR Meter was produced by American ABI –2400. Real-time PCR Detection System: Model: FOD-SSA, Primer Sequence: Analyzing Software of Fluorescence PCR Detection System V4.0, and the software of Gel-Pro-Analyzer (Version 3.0) could be used to quantize the Western Blot Protein belt, the

software of Excel 2000 could be used to conduct statistical processing, and the software of SPSS (Version 11.0) could be used to record and analyze the data of RT-PCR.

2.2 Materials

The materials needed in the experiment are selected from the following animals: Two yaks(*Poe*phagus grunniens) at the elevation of 5000 m from Maduo County of Qinghai; two yaks at the elevation of 3300 m from the Hainan Prefecture of Qinghai; two yaks at the elevation of 2300 m from the Xining of Qinghai; two *Bos taurus domestica* living in plain areas from the Experimental Animal Center of Henan Normal University.

The materials selected are the cardiac muscle and encephalic tissue of the above-mentioned animals. The experimental animals selected for the contrast of different elevations are yaks, and the experimental animals selected for the contrast of high elevation and plain area are yak and *bos taurus domestic* (referring to Note 1). The rabbits at low elevation were procured from the Health Department of Lanzhou Biological Product Plant (The elevation of Lanzhou City, Gansu Province is 1600 m above sea level). The mammals at low elevation (Rabbits, *Ory*ctolagus cuniculus domesticus) were directly transferred to different high elevations (10 rabbits should be transferred to Xining City of Qinghai Province with the elevation of 2300 m, 10 rabbits should be transferred to Gonghe County of Qinghai with the elevation of 3300 m, and 10 rabbits should be transferred to Maduo County of Qinghai with the elevation of 5000 m.). These rabbits were slewed after 3-week breeding (The environmental temperature should all be 18℃ with the same fodder and 12-h illumination.).

2.3 Western blot determining the heat shock response of mammal's Hsp70 at different elevations

The cardiac muscle of yaks at the elevations of 5000, 3300 and 2300 m and the *Bos taurus domestica* living in plain areas was taken after separate slaying. Partial cardiac muscle of the Hsp70 was taken, and immediately put into the buffer solution of 4℃ (40 mmol/L NaCl, 20 mmol/L Tris-HCL, pH7.5, the ratio of buffer solution = 1:10). The supernate of the homogenate was extracted after a 10-min centrifugation with the weight being 12,000 *g*[27], and the protein density was determined with the method of Neuhoff[28] through determining the protein density of the homogenate, then, the CD value of each sample at the site of 630 mm was measured with the 75-2 Spectrophotometer. The protein molecular weight was 72 kD. The density of each sample (namely the protein density) was converted, and the volume of sample was calculated according to the protein density and the total protein volume of the sample. The polyacrylamide gel electrophoresis (SDS-PAGE) was conducted with the Laemmli Method[29]. The Western blot (WB) was conducted with the Towbin Method[30]. The first antibody was monoclonal antibody of rat-anti Hsc70/Hsp70, and the second antibody was the sheep-anti-mouse IgG marked with horseradish peroxidase, and it was terminated and fixed with TE-Buffer (Tris-HCL 10 mmol/L, EDTANa 21mmol/L) after lucifugal coloration.

2.4 Determining the gene expression of mammal's Hsp70 at different elevations with RT-PCR

Yaks at the elevations of 5000, 3300 and 2300 m and the *Bos taurus domestica* living in plain areas were separately slain to select their encephalic tissue. Primer length: 23bp TM: 59 GC: 43%. Sequence of Amplified Hsp Primer (based on the pathogen needed to examine the specific gene that was being waited for examination):

upstream primer: 5′ AAA AAC ATg gCT ATC ggC ATC GA-3′;

downstream primer: 5′ CAg ATC AAA gAT gAg CAC gTT-3′.

(1) It was conducted according to the Neton Method [31,32] to extract the total RNA of cell, determine the purity, the content and the integrality of RNA, as well as operate the RT-PCR. 0.2 g of the frozen-storing encephalic tissue of yak at high elevation was put into the Trizole Reagent Box, the liquescent nitrogen was ground rapidly, and 1 mL of Trizole reagent was added in, and succussed. Then, 200 μL of chloroform solution was added in, and succussed. Then, supernate was extracted, and 500 μL of isopropanol was added in, and centrifugated. After 20 min of deposition and 20 min of centrifugation, the supernate was abandoned.

10DEPCddH_2O, and 2 μL of RNA was extracted to conduct electrophoresis, and another 2 μL of RNA was extracted to complete cDNA, and the products were stored at the temperature of –20℃ for standby.

(2) PCR of the hsp and *β*-actin genes (51 μL). The PCR amplification was conducted based on the template of the prepared total cell cDNA, and the parameters of PCR responses are 5 min of 94℃ degeneration, 94℃30s, 55℃30s and 72℃30s with total 30 circulations, and 7 min of 72℃ will be extended again. After the response was finished, 5 μL of product could be taken to conduct electrophoresis in 10 g of agarose gel. And then, the correctly identified PCR product segment was recovered with the DNA gluey reagent box, and the photograph of the product segment was taken as well.

(3) Analysis of software (the Software of One-Dsan2.03).

(4) The PCR Product of Hsp was 600 bp, and the PCR Product of β-actin was 420 bp.

2.5 Determining the difference of gene expression of mammal's Hsp70 at different elevations with fluorescence real-time RT-PCR

(1) The encephalic tissue of the yaks at the elevation of 5000 m and 3,300 m and the *Bos taurus domestica* was selected separately, and the sequence of the Hsp Amplifiesd Primer was designed through inquiring: upstream primer: 5′ agg aga tct cgt cga tgg tg-3′; downstream primer: 5′ gat gat cct cag cac gtt ca-3′.The size of the amplified product: 170 bp. The sequence of the *β*-actin gene primer: upstream primer: 5′ cac aga gcc tcg cct ttg cc-3′; downstream primer: 5′ gac cca tgc cca cca tca cg-3′.The size of the amplified product: 200 bp

(2) It was conducted according to the Neton Method[31,32] to extract the total RNA of cell, determine the purity, the content and the integrality of RNA, as well as operate the RT-PCR (referring to 2.4 (1))

(3) The two kinds of cDNA were mixed with the same volume, and then, 10 times gradient dilution was conducted as the relatively standard sample. And the density was defined as 1.0×10^{-1}, 1.0×10^{-2}, 1.0×10^{-3}, and 1.0×10^{-4}.

(4) Experiment on quantitative PCR (25 μL system). cDNA: 2 μL; primer (20 μmol/L): 0.5 + 0.5; SYBR-Green Real-time PCR master mix:12.5; DD Water: 9.5. Note that two parallels were made for each sample, and two blank controls were established as well.

(5) PCR of *Hsp* and *β*-actin genes (25 μL). PCR amplification was conducted based on the template of the prepared cell cDNA of *β*-actin gene and the *Hsp* gene, and the parameters of PCR responses are 2 min of 94℃ degeneration, 94℃30s, 55℃30s, 72℃40s, and the response will be finished with the total 38 circulation.

(6) Statistical analysis of the experimental results. The result analysis was conducted by applying analysis software. Namely, the V4.0 Software of the Fluorescence PCR Examination System was applied to analyzing the result.

2.6 RT-PCR determining the difference of the rabbit Hsp gene expression at low elevation resulting from high elevation (hypoxia)

RT-PCR was used to determine the gene expression of its cerebral Hsp70.Upstream primer: 5′ tgttgcaac catgaaggtg-3; downstream primer: 5′ aatcatccgca gaatacg-3′.

(1) It was conducted according to the Neton Method[31,32] to extract the total RNA of cell, determine the purity, the content and the integrality of RNA, as well as operate the RT-PCR (referring to 2.4(1)).

(2) The steps for extracting RNA refer to the specification (the Trizol Extracting Method).

(3) Reverse transcription: PCR of *Hsp* and *β*-actin gene (25 μL). The PCR amplification was conducted based on the template of prepared cell cDNA of the *β*-actin gene and the *Hsp* gene, and the parameter of PCR responses are 2 min of 94℃ degeneration, 94℃30s, 55℃30s, 72℃40s, ant the response will be finished with the total 35 circulation.

(4) Electrophoresis and photograph.

(5) The statistical analysis of the experimental result. The result was analyzed by applying the analyzing software (Software of One-Dsan2.03). The PCR product of the Hsp was 800 bp, and the PCR product of β-actin was 200 bp.

3 Results

3.1 Western blot determining the Hsp70 Heat Shock Response of the mammals at different elevations

Western blot was used to examine the Hsp70 expression of yaks. It is shown in Fig.1 that the stress reaction of the yaks at elevation of 3300 m is medium (namely the Hsp70 expression is medium.) in the contrast of the Hsp70 stress reaction of yaks at different high elevations, the stress reaction of the yaks at the elevation of 5000 m is drastic (namely the Hsp70 expression is the highest.), and the stress reaction of the yaks at the elevation of 6000 m is weakened (namely the Hsp70 expression disappeared). Meanwhile, there is no Hsp70 stress reaction for the *bos taurus domestica* living in plain areas. Fig.2 is the internal reference of β-actin (80 μg of sample quantity)

According to Fig.1, we find that the density of protein belt, the width of protein belt, and the color deepness of protein belt in the cardiac muscle of the yaks at different elevations are completely different. Therefore, we could confirm that their relative values are different, and the difference reflects the different Hsp expressions.

It is obvious that the effect is relatively good while the feeding of the sample is 80 μg referring to Fig.2. After screening the sample feeding, it is confirmed that the sample feeding is 80 μg while determining the Hsp expression of the yak cardiac muscle.

3.2 Determining the Hsp70 gene expression of mammals at high and low elevations with RT-PCR

(1) The quality of the total RNA in the extracted Cell. After electrophoresing the cultural total Hsp70 cell RNA of the encephalic tissue of yaks at high elevation in 1% agarose gel (referring to Fig.3), both the two belts of 28S and 18S could be clearly seen. This proves the complete quality of the extracted RNA without degradation.

(2) Determining the Hsp70 gene expression of mammals at high and low elevations with RT-PCR. Gene could be amplified based on the template of DNA using the ordinary PCR method. And it could be solved through cloning the cDNA produced by the reverse transcription of mRNA.

A: The cDNA should be synthesized through the AMV reverse transcriptase of the pdN6 random primer based on the template of the total RNA of the encephalic tissue of the yaks at high elevation, and the *Hsp70* genes of the encephalic tissue of the yaks at high elevations could be obtained through examining the PCR amplification product with agarose gel electrophoresis (referring to Fig. 4) adopting the RT-PCR reaction of pfu-DNA polymerase with the total DNA molecular weight being 540 bp.

B: The RT-PCR reaction should be conducted with the template of the total RNA of the encephalic tissue of *Bos taurus domestica* living in plain areas, and the

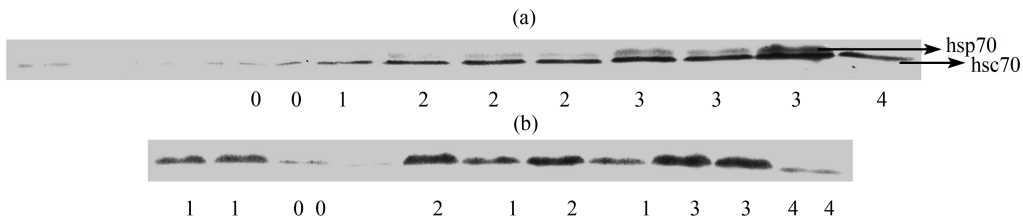

Fig. 1.(a) The Hsp70 expression of the yaks separately at the elevation of 5000 m and 3300 m and the *Bos taurus domestica* living in plain areas; (b) the Hsp70 expression of the yaks separately at the elevation of 5000 m, 3300 m and 2300 m.0, the *Bos taurus domestica* living in the plain area;1, the yak at the elevation of 2300 m; 2, the yak at the elevation of 3300 m; 3, the yak at the elevation of 5000 m; 4, the yak at the elevation of 6000 m.

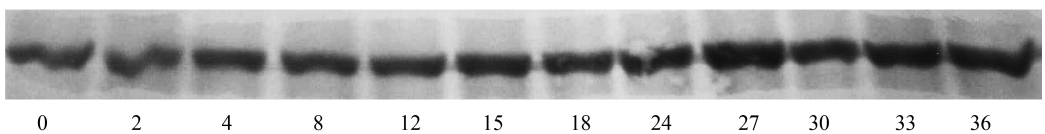

Fig. 2. β-actin (sample feeding 80 ug).

Hsp70 gene of encephalic tissue of the *Bos taurus domestica* could be obtained through examining the PCR amplification product with agarose gel electrophoresis (referring to Fig. 5) with the total DNA molecular weight being 600 bp.

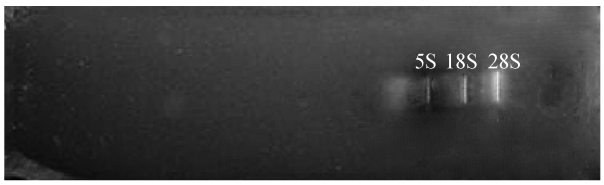

Fig. 3. Total Hsp70 cell RNA of the encephalic tissue of yaks.

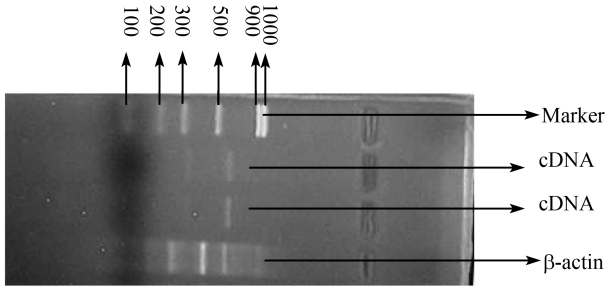

Fig. 4. DNA molecular weight being 540 bp.

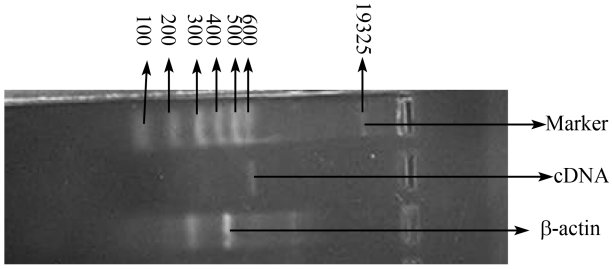

Fig. 5. DNA molecular weight being 600 bp.

C: β-actin is used to prove the correction of the cDNA, and the Marker indicates the standard of molecular weight.

3.3 Determining the differences of the Hsp70 gene expression of mammals at different elevations with fluorescence real-time RT-PCR

Since the logarithmic value of the CT value and the preliminary copy-quantity of the template becomes linear relation, the smaller the CT value, the more the targeted copy-quantity of the template, and the bigger the quantity of gene expression.

(1) β-actin: The original data (Figs. 6, 7, 8; Table1).

(2) Original data of *Hsp* gene: (Figs. 9, 10, 11; Table 2).

(3) Rectification of results.

(i) The value of the two sample *Hsp* genes shown in the Standard Curve after real-time PCR determination is 1.93×10^{-2} and 3.33×10^{-3}.

(ii) The value of the two samples internal-referring gene (β-actin) shown in the Standard Curve after real-time PCR determination is 6.56×10^{-3}, 4.99×10^{-3}.

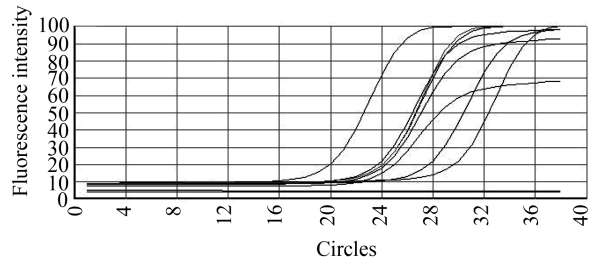

Fig. 6. The dynamic curve for β-actin determining the standard template and fluorescence real-time determining PCR.

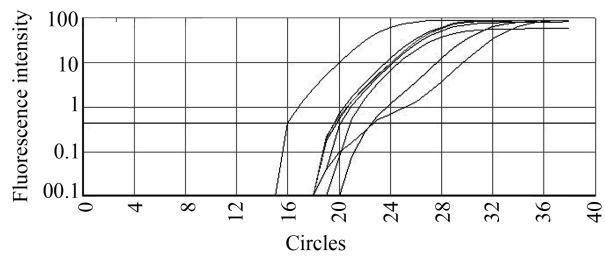

Fig. 7.

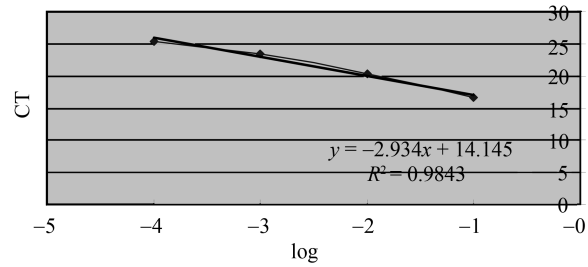

Fig. 8. Standard curve determined with the β-actin fluorescence PCR method.

Table 1 Result of β-actin expression

NO.	Name	Type	Ct	Given conc(copies)	Calc conc	%var
1	β-actin standard1	standard	16.74	1.00×10^{-1}	1.12×10^{-1}	1.2%
2	β-actin standard2	standard	20.24	1.00×10^{-2}	9.63×10^{-3}	3.7%
3	β-actin standard3	standard	23.51	1.00×10^{-3}	1.00×10^{-3}	0.0%
4	β-actin standard4	Standard	25.43	1.00×10^{-4}	1.05×10^{-4}	0.5%
5	1	unknown	20.55		6.56×10^{-3}	
6	2	unknown	20.72		5.74×10^{-3}	
7	3	unknown	20.90		4.99×10^{-3}	
8	4	unknown	20.85		5.18×10^{-3}	
9	NTC					

1 and 2 separately indicate the sample of high elevation (5000 m), and 3 and 4 separately indicate the sample of low elevation (2300 m).

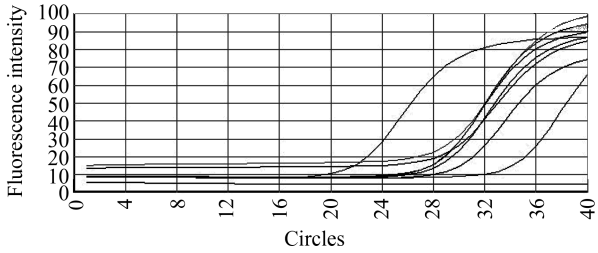

Fig. 9. Dynamic curve of *Hsp* gene fluorescence real-time determining PCR.

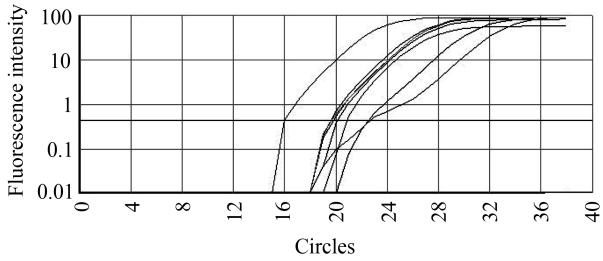

Fig. 10.

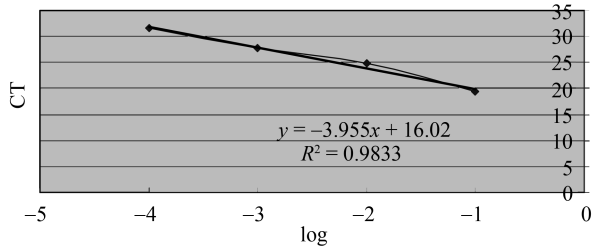

Fig. 11. Standard curve.

Table 2 Result of Hsp expression

NO.	Name	Type	Ct	Given conc(copies)	Calc conc	%var
10	Hsp standard1	standard	19.37	1.00×10^{-1}	1.02×10^{-1}	0.2%
11	Hsp standard2	standard	24.87	1.00×10^{-2}	1.01×10^{-2}	0.1%
12	Hsp standard3	standard	27.82	1.00×10^{-3}	9.62×10^{-4}	3.8%
13	Hsp standard4	standard	31.57	1.00×10^{-4}	1.13×10^{-4}	1.3%
14	1	unknown	22.80		1.93×10^{-2}	
15	2	unknown	22.65		2.11×10^{-2}	
16	3	unknown	25.82		3.33×10^{-3}	
17	4	unknown	25.67		3.63×10^{-3}	
18	NTC					

1 and 2 separately indicate the sample of high elevation (5000 m), and 3 and 4 separately indicate the sample of low elevation (2300 m).

(iii) The sample feeding of the two samples was 2 μL during the real-time PCR determination. However, due to the influence of the quantitative error of RNA density and the error of RNA reverse transcription, the cDNA contents of the 2 μL volume of each sample were different. In order to rectify the difference, the steward gene, β-actin, was used as the internal reference (the expression of different samples is basically constant). Then the value of the waiting-for-examining gene of the sample divided the value of the sample internal reference, and the final specific value was the relative content of the waiting-for-examining gene of each sample.

(iv) The specific value between Sample 1 and Sample 2 was higher than the specific value between Sample 3 and Sample 4, and this indicated that the *Hsp* gene expression of the yaks at high elevation was relatively high. Due to the high elevation, hypoxia, and the atrocious environment, the heavy *Hsp* gene expression of yaks was promoted.

3.4 RT-PCR determining the difference of rabbit Hsp gene expression in low elevation induced by high elevation (hypoxia)

(1) After electrophoresing the extracted total cell RNA and the total Hsp70 cell RNA of rabbit encephalic tissue induced by high elevation in 1% agarose gel (referring to Fig. 12), both the two belts of 28S and 18S could be clearly seen. This proves the complete quality of the extracted RNA without degradation. β-actin is used to prove that the cDNA is correct, and Marker indicates the standards of molecular weight.

(2) Twice determinations of the *Hsp70* gene expression of the heat shock response of rabbit at low elevation induced by high elevation (hypoxia) (referring to Fig.13).

4 Discussion

This research included ecology, molecular biology and plateau medicine, and the phenomena of ecology and plateau medicine were studied with the methods of immunology and molecular biology. The animal experiment showed that mammals (human beings) can not accommodate themselves with the new environment after suddenly arriving at the high-elevation area, accompanying with hard breath and other symptoms and even death, but these symptoms will be reduced after several-day rest in the secondary-high-elevation area, and then the un-adaptation of high elevation will be overcome.

1. The pathway from high elevation to low elevation (natural hypoxia)

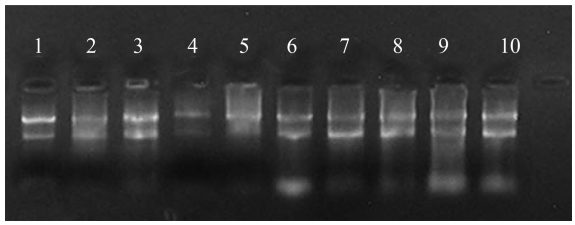

Fig.12. Total RNA of Hsp70 cell of rabbit. It indicates the RNA from 1 to 10, and both the two belt of 28S and 18S are obvious.

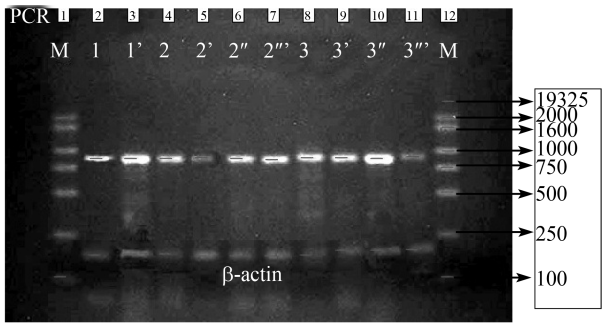

Fig. 13. Hsp70 Gene Expression of the heat shock response of rabbit at low elevation induced by high elevation. The series of 1 indicate the elevation of 2300 m, the series of 2 the elevation of 3300 m, the series of 3 the elevation of 5000 m, and M indicates the marker. It indicates that β-actin is from 2 to 11. The PCR product of Hsp is 800 bp, and the PCR product of the β-actin is 200 bp. It should be determined twice that the Hsp70 Gene Expression of rabbit heat shock responses at low elevation induced by high elevation (hypoxia).

Table 3 The rectification of results

Name of sample	Sample of high elevation		Sample of low elevation	
Serial number	1	2	3	4
Beta-actin (materials for internal reference)	6.56×10^{-3}	5.74×10^{-3}	4.99×10^{-3}	5.18×10^{-3}
(*Hsp*)gene waiting for determining	1.93×10^{-2}	2.11×10^{-2}	3.33×10^{-3}	3.63×10^{-3}
Value after rectification (*Hsp*/*β*-actin)*	2.94	3.67	0.67	0.70

The specific value between 1 and 2 is higher than the specific value between 3 and 4, and this indicates that the Hsp Gene Expression of the yaks at high elevation is relatively high. Due to the high elevation, hypoxia, and the atrocious environment, the heavy HspGene Expression of yaks is promoted.

Table 4 The different heat shock gene expression of low elevation rabbit induced by high elevation (hypoxia) ($\bar{x}\pm s$)

Number of groups	Original place	Elevation (m)	Expression of Hsp70
1st Rabbit group	Xining City	2300	0.16 ± 0.14
2nd Rabbit group	Gonghe County	3300	0.14 ± 0.12*
3rd Rabbit group	Maduo County	5000	0.12 ± 0.14*

The ratio between the 3rd rabbit group, the 2nd rabbit group and the 1st rabbit group is as *P<0.05.

2. The pathway from low elevation to high elevation (induced hypoxia)

The test proves the following facts: heat shock protein is the substance produced by organism to stress reaction, and the heat shock protein promotes the organism to accommodate the high-elevation stress. And the expression of high-elevation stress to the induction of Hsp70 is drastic. Both the mammals at high elevation and the mammals at low elevation have the gene of heat shock response with different response extent. The gene expression of heat shock protein will appear while mammals are arriving at high elevation from low elevation. Therefore, it is proved that heat shock pesponse is a form of mammals' accommodating themselves with the high elevation (hypoxia).

Different Hsp70 expressions have been discovered in the yaks at different elevations through determination with Western blot. Hereinto, the Hsp70 expression of the yaks reached the most at 5000 m, and gradually decreased at 3300 m but did not have obvious difference at 3300 m and 2300 m. Additionally, the capacity of synthesizing Hsp70 of the yaks at the elevation of 6000 m is decreased. The capacity of synthesizing Hsp70 of the *Bos taurus domestica* living in plain areas is normal. The differences of Hsp70 expressions of the yaks at the elevation of 5000 m and the *Bos taurus domestica* living in plain areas are obvious. The result of the experiment showed that high elevation (hypoxia) could obviously increase the Hsp70 expression in the cardiac muscle of yak, but there was no Hsp70 expression in cardiac muscle of the *Bos taurus domestica* living in plain areas.

It is discovered that the mammals at different elevations all have the Heat Shock Response Gene that will be activated while the stress reaction through the determination of RT-PCR and Real-time PCR Detection. Through twice RT-PCR determination that the mammals at low elevation directly arrive at different high elevations, these experimental mammals will show the heat shock response to different extent under the induction of hypoxia, and the heat shock protein gene of cade rabbits start to express. The experimental result shows that the fast expression of Hsp70 is suitable for cells maintaining normal physiological functions, and threshold may exist in the Hsp70 expression of heat shock response. The heat shock response of the yaks at the elevation of 5000 m is the best heat shock condition, and the heat shock response of the yaks at the

elevation of 6000 m gradually decreases. The capacity of mammals accommodating the high elevation is formed gradually, which indicates that the heat shock response begins to produce when the elevation reaches 2200 m, and the production of heat shock response reaches the fastigium at the elevation of 5000 m, but the production begins to decrease exceeding the elevation of 6000 m. The special result proves that the higher the elevation is, the more atrocious the hypoxia is, and the more obvious the Hsp70 expression of organism is. However, if the elevation exceeds a certain height, the protection function of Hsp70 will disappear. The research on *drosophila* proves that through determining the Hsp70 content of drosophila cells in heat shock response and in normal condition with Hsp70 monoclonal antibody, the increment of Hsp70 in drosophila cells during the heat shock response could reach 1000 times of the increment of Hsp70 during normal condition[33].

Along with the enhancement of the Hsp70 expression, the capacity of organism tolerating heat is rapidly increased as well. Xiong discovered that both the Hsp70mRNA gene expression and the synthesis of Hsp70 protein are obviously increased under high temperature through the research on rats[34]. It is obvious that the *Hsp70* gene is activated under the condition of heat stress, and the RNA polymerase of the *Hsp70* gene activates the promoter of *Hsp70* gene under the adjustment of transcription regulator factor, then plenty transcription Hsp70mRNA synthesizes *Hsp70* protein, all these result in the increase of the Hsp70 expression. However, the protection of Hsp70 to cells is not unlimited, and the protection cannot be illimitably exaggerated. Wang *et al*. discovered that the protection could only protect cells to a certain extent when the stress exceeded a certain extension and cells decrepitated at 45℃ for wore than 6 h, no matter the cells have high Hsp70 expression or low Hsp70 expression, the activation of cells would decrease [35].

The reason is that if the time of stress is too long, the synthesis of the protein that could maintain normal growth of cells will be stopped to threaten the survival of cells. Meanwhile, too much production of denatured protein will overrun the protection of Hsp70, and the function of cells will be influenced to result in the death of cells. After organism is affected by blight environment, the normal protein gene expression will be automatically closed or decreased, and the Heat Shock Gene will be activated to produce Hsp. Therefore, it is the essential mechanism to know the production of Hsp70 to understand the transcription of *Hsp70* gene[36]. The adjustment of *Hsp70* gene expression includes basic expression and induced expression, and it is the best model to research the adjustment of gene transcription[37]. During the heat shock response, the change of Hsp70 mainly depended on the extent of transcription and translation[38], and is closely connected with the activation of the Heat Shock Transcription Factor, Hsf. Additionally, the Hsf is various, and Hsf1 is the most closely connected with the intracorporeal and extracorporeal stress. It is a process with several steps to activate Hsf1, and the basic process is as follows: Stress→ Tri-polymerization of Hsf1→The Trimer of Hsf1 transferring to the nucleus from cytoplasm → Syntheses of Hsf1 and Hse → Phosphorylation of Hsf1 Serine Residue→Promoting the Transcription of the *Hsp70* gene and so on [39].

The activation of Hsf is rapidly changed under different temperatures during the period of cultivating in the laboratory, and HSF plays an important role in the heat shock response[40]. The synthesis of Hsp70 is increased while cell stress and the synthesis of other protein are decreased[41]. It becomes the focus of the recent researches on how to effectively induce the Hsp70 expression, especially the research on how to induce or latently induce the synthesis of Hsp without any lesion of cells has great application prospect. Some literatures report that the tolerance to hypoxia of organism at high elevation is distinctly enhanced[42], and thus the time of organism tolerating the hypoxia could be extremely lengthened[43]. Hsp is a kind of highly conservative protein with its unique biological characteristics and functions, and pervasively participates in various complicated functional activities of organism. People compare it to a *Swiss Saber*[44], this comparison more visually reflects its polychrest biological effect.

At present, many researches have proved that the form of Hsp is evolving, for instance, Rutherford, *et al*.[45] thought that Hsp could buffer the phenotypic changes of species. Namely, natural selection could increase the genetic polymorphism of species, and the

phenotypic changes of organism would not appear immediately under the adjustment of Hsp until the environment of species is stressed. Accordingly, when the organism is stressed, neutral mutation could be changed into elective mutation by some mechanism in which the Hsp participates to accommodate the necessity of selection. In other words, Hsp could influence gene expression under the pressure of selection, through adjusting the correct fold of mutant protein, or indirectly adjusting signal to buffer the changing phenotype, thus the form-gene that is not suitable for the environment will not be expressed immediately, but the cryptic advantageous gene will be expressed, but if the gene cannot accommodate the environment, death will result from it. Through experiment, Lerman[20]*et al*. proved that when the environment was changed, the genetic material of Hsp would produce quantitative changes, and the changes would make the species different, and natural selection would affect them. The research on this of Emily Stenseng, *et al*. [46] is still continued, and the research has become one of the focuses of molecular biology.

The theory of Feder [47−54] is as follows:

(a) So far, the result of the research on heat shock protein is the best evidence: adequate heat shock protein could influence the adaptation of a wild animal.

(b) The physiological significance of heat shock protein is to remain the level of the overall organism, especially in the complicated eukaryotic cell. heat shock protein is a factor to evaluate the background role of other unique characteristics. Furthermore, the modern molecular technology provides an unprecedented opportunity to operate gene background of an unique characteristics opposing the constancy in order to evaluate the significance of characteristics.

(c) The influence of Hsp70 to *drosophila* focuses on the changes of copied numbers of the whole larva and chrysalides. Contrarily, the tolerance of Heat Shock will be influenced to potentially impact the adaptation of *drosophila*.

(d) The research on Heat Shock Protein has very important significance, since it indicates that the first usage of genetic program is to be applied into the research on zoo-ecology and physiological evolution as a tool.

(e) Many previous researches show that it is accordant between the significances of the interacted Heat Shock Response and Adaptation. And the expression of Hsp70 proves that the research on Hsp70 still is a challenge to the future. The Heat Shock Protein is the pressure indicator of biochemistry, and Hsp could be induced by environmental pressure[55].

The actual significance of the research is that all of the mammals (including human beings) have *Hsp* Gene, and if only the mammals could go to the high-elevation areas from the low-elevation areas slowly and gradually, the body could accommodate to the environment, namely, the body will occur the heat shock response to produce Hsp. However, the adaptation still has its limit (e.g. the elevation of 6000 m in the test), and mammals cannot tolerate and survive exceeding the limit. Therefore, the ecological significance of the research is as follows:

(a) It will be explored that whether the same biology will start different Hsp to the stress of different environmental ecological factor, and it will be explored that whether different biology will start different Hsp to the stress of the same environmental ecological factor as well.

(b) Hsp will be explored as the indices for evaluating the capacity of special people accommodating the environment (such as the capacity of mountaineers accommodating high elevation and hypoxia, as well as the capacity of divers accommodating the hypoxia in deep sea).

(c) It should be connected with the studied biome and the stimulation of the environmental ecological factor to explore the functions of Hsp.

(d) It will be explored that a technical measure could enhance the Hsp expression of organism, and the measure could be applied to the environment under the effects of different ecological factors to enhance the anti-reverse capacity of organism.

(e) A strong revulsant with no lesion (or little lesion) will be explored in order to induce organism to produce Hsp, therefore, the organism could be protected from the injury of atrocious environmental ecological factors.

(f) Human beings could only survive in the nature through complying and protecting the nature, and the rules that are propitious to the human beings could be discovered in the nature in order to achieve better ad-

aptation to the nature. The research will provide scientific academic foundation for human beings to accommodate the plateau.

Note 1: Mammals that are at high-elevation areas are mainly yaks. At present, the domestic researches to yaks are mainly focused on improving the anti-reverse of yak breed and adjusting the structure of yak herd, and the researches to *Bos taurus domestica* are mainly focused on the hybrid improvement of the four breeds of *Bos taurus domestica* to select high-quality breeds of Chinese beef cattle and milch cow. There is no genital isolation between yak and *Bos taurus domestica*, and the kinship seems between genera, and the kinship is little more distant related than ordinary genera. However, the difference between genera cannot be eliminated. Therefore, *Bos taurus domestica* could only be the representative of the Hsp70 expression of the mammals living in plain areas. It has a long history to hybridize yak and *Bos taurus domestica* in China, and buffalo is utilized to hybridize with common cattle (i.e. beef cattle) in the United States and Canada with lots of experiments. If the kinship between the genera is too distant, the hybridization will not succeed due to lacking of chromonemal identity. For instance, it cannot be fertilized after the mating between water buffalo and *Bos taurus domestica* [56−58].

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