GLOBAL JOURNAL OF BIO-SCIENCE & BIOTECHNOLOGY

www.scienceandnature.org

ISOLATION, PURIFICATION AND KINETICS OF DEOXY RIBONUCLEIC ACID AT DIFFERENT STAGES OF FEEDING IN *BOMBYX MORI*

[a]Brindha S. , [b] Maragathavalli S., & [c]Gangwar S. K., & [c] Annadurai B
[a]Department of Bioinformatics, New Prince Arts and Science College, Chennai,
[b]Department of Biochemistry, King Nandi Varman College of Arts and Science, Thellar,
[c]Biotechnology Team, College of Dryland Agriculture and Natural Resources, Mekelle University, Mekelle, Ethiopia.

ABSTRACT
The recent explosive progress in understanding the gene structures and function has depended upon the methods of detection of small amounts of nucleic acid. It is confirmed, on identification of gene which is obtained. It helps in analyzing the structure, function and expression of the gene. The application of the isolation, is for structural analysis and functional analysis of the desired gene.They are incorporation of label to monitor enzymatic reaction, processing of nucleic acids and hybridization experiments. The interaction of the protein and nucleic acid play a major role in all aspects of gene expression. Whether one is considering replication starting with RNA priming followed by the mechanism of action of DNA polymerases and the various replication proteins. There are as well the structural proteins involved in packing mechanism such as the organization of DNA in to a chromosome are attachment of the chromosome to the cytoskeleton matrix. The protein and DNA interaction follows the following pattern of investigation To locate the region of DNA molecule where the protein interact, to localize the protein binding region on the DNA fragment to the exact area of protein - DNA contact, determine which actual bases and phosphates on the DNA are involved in the interaction with the protein and three dimensional conformation of the DNA-protein interaction by the means of x-ray crystallographic analysis. In this study an attempt has been made to estimate protein at each stage of the *Bombyx mori* larva since silk is a protein. Protein in silk gland and at each stage of larva is quantitatively estimated. Since nucleicacid are the causative factors for secretion of proteins quantitative estimation of DNA and RNA at different stages of silkworm were found out and presented. The protein in larva and silk gland, DNA and RNA in larva and silk gland were separated and presented with the help of PAGE, SDS-PAGE, and PFGE respectively. It is a pioneering work in this interesting field of sericulture industry.

KEY WORDS: Isolation, purification, *Bombyx mori*, DNA protein, nucleic acid, PAGE, SDS etc.

INTRODUCTION

The word silk spells luxury and class. No other fabric in the world can match silk for its luster and eligance. The touch of silk on the finger tip evokes, the very thread of history, a shimmering fabric of and paralled grandeur. For the past several centuries it has raised an undisputed as the queen of textiles. Mankind has always loved silks. It is the yarn of life, extruded by that unassuming catterpillar in a continuous filament as long as nearly one and half kilometers.

Silk provides much needed work in several developing and labour rich countries. More than 10 million farmers raise silk in China today. India now the second largest silk producing country in the world next only to China and provides employment for over 5 ½ million people.

The Indian silks are known for their finery. The masterly brocades of Banaras and Surat, the soft-as-a-sigh silks of Karnataka, tie and dye magic of the Patola from Gujarat are famous throughout the world. Then there are the ikats of Orissa, the delicate silks of Kashmir, shear brilliant fabrics of Bandhej and the temple silks of Kancheepuram and Tanjore in Tamil Nadu were master craftsmen blend skillfully the art of India with the smoothness of the silk yarn to produce works of supreme creativity

Silk

Silk is a pasty secretion of silkworm around it. Silk worm is the caterpillar of silk moth *Bombyx mori*. The silk is secreted by a pair of silk glands, the modified salivary glands. The caterpillar secretes silk fiber on the 42nd day. It gives on spinning silk around it for 3 to 5 days to produce a silky coat called cocoon. The cocoon is male up of a single continuous thread.

Silk is made up of two types of insoluble proteins namely fibroin and sericin. Fibroin constitutes 80% and sericin constitutes 20%. Chemically silk, thread is made up of a fibrous protein, insoluble in water. The silk thread has an inner core of fibroin surrounded by sericin. Molecular formula of fibroin is C_{30} H_{46} N_{10} O_{12}. The molecular formula of sericin is C_{30} H_{40} N_{10} O_{16}. Fibroin of silk is a β - Keratin.

Silk fibroin and other β- Keratin have a very high content of glycine and alanine, the amino acids with the smallest R groups. In deed in silk fibroin every other amino acid is glycine. The polypepride chains in silk fibroin exhibit β - conformation. In the β - conformation, the backbone of the polypeptide chains is extended into zigzag rather than a helical structure. In silk fibroin the zigzag polyperptide chains are arrange side by side to form a structure resembling a series of plates. Such a structure is called a pleated sheet (Quan *et al*., 1998).

In the β - conformation there are no interchain hydrogen bonds instead these are no interchain hydrogen bonds between. The peptide linkages of adjacent polypepride chains. All the peptide linkages participate in such interchain hydrogen bonding. The R groups of the amino acids protrude out from the zigzag structure as seen in the edge view.

There are no cystine cross linkages between the side-by-side chains of β-karatins unlike that of α-keratins. In silk fibroin, adjacent polypepride chains are usually oriented in opposite or antiparalled directions with the amino terminals at opposite ends.Three zigzag polypeptide chairs of silk fibroin are arranged side by side to form a pleased sheet.

GENETIC STOCKS OF THE SILKWORM

A large number of inbred and characteristic stocks are maintained more than 320 herditory trairs of the silkworm there so far been analysed. Most of them are related to morphologically recognizable characteristics silkworm genes are classified into groups according to the different developmental stages they affect.

Normally the silkworm egg is short-elliptic, slightly narrowed at the anterior, and has dark brown pigment in a single-layer membrane of serosa. The egg shape is entirely dependent on the shape of the chorion which is formed in the maternal body before fertilization. The serosa, tying underneath the chorion and covering the yolk and embryo, consists of cell which is derived from cleavage nuclei. The abundance of egg colour mutants due to serosa pigment is a unique feature of silkworm genetics. The normal egg is dark brown, white mutants are white, pink, red, brown and so on. Pigment in serosa cells is light orange; the compound eyes at the moth are pink.

AIM AND SCOPE OF THE WORK

Human beings of our earth are unique among all living organism on earth. He only can think and develop complicated languages that allow meaningful and complex interplay of ideas and emotions.He only as a supreme of all living organisms can use,can display, can dissect and can analyse all livingthings on earth for his use. *Bombyx mori* is one such organism, which has been interacted, interrelated and interwoven together with the life of our lives. Silk is liked by all women irrespective of the age and position young or old or servant maid or Queen.India is one of the rich country learned and made other countries to use silk.Vast researches has been done on silk on the aspects of its feed namely Morus alba.Many researches have been carried out on the morphology, Anatomy, cytology, Embryology and Physiology of Bombyx mori. But the molecular biology of silkworm yet to start its beginning in this field.The evolution of genes is complex and it is sub discipline of our biology.

Recent explosion of human genome sequence analysis by the American molecular biology enthrilled the world of the discovery of death sequences and disease susceptible genes and inborn errors in gene sequences will be going to prolong the life of all living organisms including man upto 1300 years.This made all scientists turned their faces towards biology.

Hence an attempt is concentrated with the suggestion of our head of the department of Zoology, PG extension centre and constant help of the staff members,the following aspects of *Bombyx mori* is tried.

1.Estimation of DNA and RNA at various stages.
2.Estimation of DNA and RNA in silkgland.
3.Isolation of DNA and RNA at various stages.
4.Poly Acrylamide Gel Electrophoresis (PAGE) of DNA and RNA at various stages of silkworm

1. .Poly Acrylamide Gel Electrophoresis (PAGE) of DNA and RNA at various stages of silkgland.
2. Pulse Field Gel . Electrophoresis (PFGE) of DNA and RNA at various stages of silkworm.
3. .Pulse Field Gel Electrophoresis (PFGE) of DNA and RNA at various stages of silkgland.

This is minor venture in this field of sericulre has been attempted and presented in this dissertation.

MATERIALS AND METHODS

Isolation of deoxyribonucleic acid (DNA)

Nucleic acids (DNA and RNA) are vital macro-molecules in all living cells. The DNA contains the basic genetic information. As such cellular DNA is located at the site of primary genetic activity (nucleus) within the cell. In prokaryotic cells, genetic activity occurs throughout the cell while in eukaryotic cells it lies in discrete particles within the cells. Most of the DNA of eukaryotes exists in the nuclei and the remaining DNA in the partially self-duplicating mitochondrial and chloroplast particles. The nuclear DNA combines with histone proteins in an orderly manner to form chromatin.

Extraction of DNA is done by a number of methods. The efficiency and recovery of extraction depends on the sample material, ionic conditions of the extraction medium, type of lysing agent used etc. The procedure described below is essentially that of Murmur's method.

Principle

Extraction of DNA is accomplished by the rupturing of cell wall and nuclear membrane followed by deproteinization and precipitation of the nucleic acid using ethanol.

Materials

Sample material

Extraction medium 0.15 M NaCl, 0.1 M Na_2 EDTA

Lysozyme solution, 10 mg/mL

25% SDS solution

5 M $NaClO_4$.

Chloroform: Isoamyl alcohol (24:1)

95% Ethanol

Saline citrate (1x) 0.15 M NaCl, 0.015 M Trisodium citrate (also 10-fold and 1/10-fold concentrations).

3 M Sodium acetate

Isopropanol

Procedure

1. Grind 2 g of the sample material in 25 mL of extraction medium in a pre-chilled pestle and mortar.
2. Add 1 ml of lysozyme solution to the above suspension and incubate at 37°C for 30 min, shaking occasionally.

3. After the incubation, complete the lysis by adding 2 ml of SDS solution, heating this preparation for 10 min in a 60°C water bath, and finally cooling the solution to room temperature in a bath of tap water.
4. Add sufficient 5 M perchlorate solution to the lysed preparation to a final concentration of 1 M.
5. Add an equal volume of chloroform-isoamyl alcohol (24:1) to the lysed preparation suspended in 1M perchlorate and slowly shake (30-60 oscillations/min) in a tightly stoppered flask for 30 min at room temp.
6. Separate the resulting emulsion by centrifuging for 5 min at 10,000 g at room temperature.
7. After centrifugation, carefully pipette off the top clear aqueous phase from the coagulated protein emulsion at the interface.
8. Place the aqueous phase containing the nucleic acids in a beaker.
9. Gently stir the nucleic acid solution with a sterilized glass rod while slowly adding two volumes of 95% ethanol down the side of the beaker so that ethanol is layered over the viscous aqueous phase. Continue to gently stir the preparation to mix ethanol throughout the entire aqueous phase and spool all of the gelatinous, thread-like DNA rich precipitate on the glass rod.
10. Drain off excess fluid from the spooled crude DNA by pressing the rod against the wall of beaker until no further fluid can be squeezed from the spooled preparation. (If the squeezing is not done sufficiently, the alcohol adhering to DNA will make it difficult to dissolve DNA).
11. Dissolve the crude DNA on stirring the glass rod with its spool of material in 9 ml of dilute (1/10 fold) saline citrate in a test tube or small beaker. If any solubility difficulty is encountered, continue working the sample to obtain an even suspension.
12. To the even suspension, add 1 ml of 3 M sodium acetate, 1 mM EDTA, pH 7.0 solution, transfer the preparation to a 100 mL beaker, and gently swirl the sample while dripping in 5.4 ml of isopropanol.
13. If fibrous DNA is readily apparent, collect the DNA threads by stirring and spooling with a sterilized glass rod as before. If a gel-like preparation develops, add 0.5 ml more of isopropanol and stir to spool the DNA threads as before. Finally, remove excess fluid from the spooled DNA by pressing the sample against the walls of the beaker.
14. Wash the sample in test tubes containing, in turn, 10 ml of 70% ethanol and then 10 mL of 95%. Store the DNA in a stoppered tube (2°C refrigerator) as a spool submerged on the rod in 95% ethanol.
15. Remove alcohol from the spooled DNA by blotting with a clean piece of filter paper and then dissolve the DNA by stirring the glass rod in a test tube containing 9 ml of dilute (1/10 fold) saline citrate. When the DNA is dissolved add 1 ml of concentrated (10 X) saline citrate solution to achieve approximately a standard (1x) saline citrate concentration. This solution can be stored at 2°C with a few drops of $CHCl_3$.

Diphenyl amine test for DNA

Deoxypentoses react with diphenylamine in the presence of strong acid to form a blue compound. As DNA contains deoxyribose, it also reacts with diphenylamine. The appearance of a blue colour is a positive test for DNA.

Procedure

Take 1 ml of the DNA solution in a test tube, add 4 ml of diphenylamine reagent and heat in a boiling waterbath for 10 minutes observe the colour that appears.

DNA solution

Dissolve 26 mg of DNA in water and make upto 100 ml.

Diphenylamine

Dissolve 1 g of diphenylamine in 100 ml of glacial acetic acid and carefully add 2.7 ml of concentrated sulphuric acid store in refrigerator.

Test for nucleic acids

Nucleic acids are the important class of informational macromolecules present in all cells. The two groups of nucleic acids are deoxyribonucleic acid (DNA) and ribo nucleic acid (RNA). The nucleic acids are actually polynucleotides, the component nucleotides being linked together through phosphodiestyer bridges. The nucleotides contain purine or pyramidine bases, a sugar moiety and a phosphate group. The bases adenine, guanine and cytosine are common to both DNA and RNA, DNA also contains the base thymine, and the suar deoxyribose while RNA contains the base uracil and the sugar ribose. The qualitotive tests for, as also the quantitative estimation, of the nucleic acids are based on the specific reaction of the sugar moiety present in the compound.

REAGENTS REQUIRED

DNA solution

Dissolve 26 mg of DNA in water and make up to 100ml. RNA solution. Dissolve 20 mg of RNA in 100ml of hot 5% Trichloroacetic acid.

Diphenyalanine

Dissolve 1g of diphenylamine in 100ml of glacial acetic acid and carefully add 2.7 ml of conc. sulphuric acid. Store in refrigerator

Orcinol

Dissolve 1g of orcinol in 100 ml of concentrated hydrochloric acid containing 0.5g of ferric chloride.

Orcinol test for RNA

This is a general reaction for pentoses which on heating with conc. hydrochloric acid undergoes dehydration to give furfural. The furfural gives a green colour with orcinol in presence of ferric chloride as a catalyst. RNA, which contains ribose answers this test.

Procedure

Take 1ml of the RNA solution in a test tube, add 2ml of orcinol reagent and heat in a boiling water bath for 20 minutes. Note the color.

Diphenylanine test for DNA

Deoxypentoses react with diphenylamine in the presence of strong acid to form a blue compound. As DNA contains deoxyribose, it also reacts with diphenylamine. The appearance of a blue colour is a positive test for DNA.

Procedure

Take 1ml of the DNA solution in a test tube, add 4 ml of diphenylamine reagent and heat in a boiling water bath for 10 minutes. Observe the colour that appears.

Ultra violet absorption

The visible spectrum of light extends from 6,500 A to 4500A. Wave length shorter than 4,500A are called ultraviolet. Nuleic acids absort ultra violet light at a wavelength of 2,600A. By this method it is possible to locate DNA without staining the chromosomes. Casperrson and others have used this method to measure the nucleic acid content of nuclei.

Isolation of DNA from adult flies

The following is used for protocol for isolating genomic DNA from adult flies which is modified slightly from that published by Bender *et al.* (1944).

Equipments and reagents

- 7 ml ground-glass homogenizer (protocol 1)
- Grinding buffer : 0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl, 0.05 M EDTA, pH 9.1, freshly added sodium dodecyl sulfate to 0.5% and diethyl pyrocarbonate to 1%
- Elutip-d column and elution buffer recipe (Schleicher and Schuell)
- RNase A buffer (Protocol 5)
- RNase A (Protocol 5)
- Centrifuge and rotor with adaptors to hold 15 ml glass tubes (e.g. sorval SS 34 rotor Du Pont).

Method

1. Collect approximately 100-200 adult flies by anaesthetizing with CO_2.
2. Transfer the flies to a 7 ml ground – glass homogenizer containing 2 ml of grinding buffer and grind quickly at room temperature.
3. Transfer the homogenate to a 15 ml thick walled glass tube and incubate at 65°C for 30 min.
4. Add 0.3 ml of 8 M potassium acetate and incubate on ice (4°C) for 30 min.
5. Centrifuge at 9100 r.p.m. (10,000 g) for 5 min. at 4°C.
6. Mix the supernatant with an equal volume of ethanol.
7. Let stand at room temperature for 5 min.
8. Centrifuge at 9100 rpm. (10,000 g) for 5 min. at room temperature.
9. Rinse the pellet with 80% (v/v) ethanol in d H_2O.
10. Dry the pellet under vacuum being extremely careful not to under or overdry.
11. Resuspend the pellet in 1 ml of low salt buffer for purification through an Elutip-d column. The DNA solution may appear cloudy due to residual carbohydrates and eye pigments.
12. Purify the DNA through the Elutip-d column according to the manufacturer's specification (eliminate the pre-filter).
13. After ethanol precipitation, perform steps 21-24 inclusive of protocol 5 and resuspend the pellet in 100 µl of RNase A buffer.
14. Add 4 µl of RNase A and incubate at 37°C for 1-3 hours.
15. Add sodium acetate to 0.2 M and then add 250 µl of 100% ethanol.
16. Precipitate the DNA on ice for 10 min or at -20°C overnight.
17. Perform steps 21-24 inclusive of protocol 5 and resuspend the pellet in 50 µl of TE buffer.

NB. The yield from 200 flies is about 60 µg of DNA.

Estimation of proteins

Protein content was determined by adopting the procedure of Lowry et al., (1951) described under general materials and methods with bovine serum albumin (BSA) as standard. The fractions collected after chromatographic column were directly read at A 280 nm in a shimadzu spectrophotometer for the presence of protein.

Statistical procedure

The mean and the standard deviations were calculated from the determined values by using the standard procedures (Bailey, 1984). The standard deviation was calculated by using the formula.

$$S = \frac{(x_1 - \bar{x})^2}{n - 1}$$

where x_1 = value of individuals
x = mean value of the sample
n = number of samples.

In order to examine whether the difference in results obtained was significant or not the following formula (students `t' test) was employed.

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{S_1^2/n_1 + S_2^2/-n_2}$$

where $\bar{x}_1$ = mean value of one sample
x_2 = mean value of other sample
S_1 and S_2 = corresponding standard deviations
n_1 and n_2 = the number of tests for each sample.
The level of significance (P-value) between x_1 and x_2 was determined by using the students `t' distribution table of fractiles and critical values (Radhakrishna Rao *et al.,* 1985).

RESULT

TABLE 1: Nucleic acid content in different stages of *Bombyx mori* Larva

S.No.	Developmental Stage	Larval weight (g)	Protein (Mg/g)	DNA (μg/g)	RNA(μ/g)
1.	I Instar	0.15	0.875	26.52	20.06
2.	II Instar	0.96	1.212	165.18	52.04
3.	III Instar	1.26	1.963	820.05	224.12
4.	IV Instar	2.12	2.043	1045.12	665.25
5.	V Instar	3.28	2.128	1526.21	1123.63

Values expressed are the Mean (X) of 6 individual experiments
df = n-1 observation
* = P<0.01
** = P<0.001
NS = Not significant

Table 1 shows the nucleic acid content, (DNA and RNA) protein content and larval weight in different stages of *Bombyx mori* larva. The larval weight is 0.15g, 0.96g, 1.26g, 2.12g and 3.28g from I instar to V instar stage respectively. The protein content is steadily increased from 0.875μg/g to 2.128 μg /g.The DNA content ranges from 26.5μg/g from I instar to 1526.21μg/g in V instar stage.The RNA content ranges from 20.06 μg/g from I instar to 1123.63 μg/g in V instar.

TABLE 2: Nucleic acid content in Silkgland

S.No.	Developmental Stage	Protein (Mg/g)	DNA (μg/g)	RNA(μ/g)
1.	III Instar	1.84	953.18	1032.67
2.	IV Instar	2.04	1265.47	1412.12
3.	V Instar	3.256	1978.32	1637.58

Values expressed are the Mean (X) of 6 individual experiments
df = n-1 observation
* = P<0.01
** = P<0.001
NS = Not significant

Table 2 shows the nucleic acid content, RNA content,and protein content in silkgland III in star to V instar stage.of silkworm larvae
The protein content was maximum in V instar stage (3.256 μg/g) The protein content is minimum in III instar stage (1.84 μg/g).The DNA content in silkgland is minimum in III instar stage (953.18μg/g) and was maximum in V instar stage (1978.32 μg/g).The RNA content in silk gland is minimum in III instar stage of *Bombyx mori* (1032.67 μg/g) and was maximum in V instar stage (1637.58 μg/g).

DISSCUSION

From Table 1 & 2 the DNA and RNA contents of silkgland and different stages of larva show, the corresponding increase of body weight and protein content. It increases the DNA and RNA content proportionately in the larva as well as in silkgland.
The tissues of silkworm were homogenized separately in ice cold water and homogenate was used for protein, DNA and RNA analysis. The estimation of total protein (lowery *et al.,* 1951) nucleic acids, (1957), DNA and RNA (Burton, 1956, and Ceriootie,1955). Higher level of protein, DNA and RNA is seen by increasing from I to V instar stage. Significant increase of silk gland, filament length, Cocoons weight was also noticed. The DNA content in insect tissue is an index for expressing other biochemical contents like Protein and RNA.The increase in DNA to RNA along with protein suggest, the activation of metabolic process like protein synthesis. It also express the protein metabolism of silkworm (Ueda,1982).

REFERENCES

Avery, O.T., C.M. Macleod, and M. Mc Carty (1944) Studies on the chemicla nature of the substance inducing transformation of pneumonococcus types. J. Exp. Med. 79: 137-158.

Bailey, T.J., (1984) Statistical methods in Biology, Second Edn. Hodder and Stoughton, London.

Burton., K., (1956) A study of the condition and mechanism of the diphenylamine reaction for colorimetric estimation of deoxyribo nucleic acids. Biochem. J. 62, 315-325.

Cerriotic, G. (1955) Determination of Nucleic acids in animal tissues. *J. Biol. Chem*. 214 – 59.

Chudinova, E.M., Zatsepina, O.V. (1998) DNA localization in nuclei of the sea urchin *paracentrotus tiridus* oocytes Biologicheskie membrany (Moscow).**15** (6): 648-656.

Crick, F.H.C., and J.D. Watson (1954)The complementary structure of deoxyribonucleic acid. Proc. Roy. Soc. (A) 223: 80-96..

Drevet, J.R., Swevers, L. (1995) Developmental regulation of a silkworm gene encoding multiple gata type

transcription factors by alternation splicing J.Mol. Biol.246 (1):43-53.

Fujiwara, Haruhiko (1995) Structural and evolutionary features of ribosomal RNA; and rDNA of *Bombyx mori*. J. Seric. Sci. Jpn. 65 (5); 401-409.

Furusawa, Tohiharu, Leslie, S., Indrasith (1985) Changes in RNA, DNA content and RNA/DNA ratios during the embryonic diapause of the silkworm *Bombyx mori*. J. Seric. Sci. Jpn. 54 (1); 53-62.

Gao, Q., and Cai, Y. (1994) Studies on the structure of nucleic acids of the desonuclevirus of *Bombyx mori* China (Zhen Jibang) strain sericologia; 34 (3): 447-450.

Griffith, F. (1928)The significance of pneumonococcal types. J. Hyg. 27: 113-159.

Hershey, A.D., and M. Chase (1952) Independent function of viral protein and nucleic acid on growth of *bactriophage. J. Gen. Physiol*. 36:39-56.

Higuchi, R., Bowman, B., Freiherger (1984) Natre 312, 282-284.

Holley, R.A. (1965) Mechanism for gene conversion in fungi. Genet. Res. 5, 282.

Islam, S., Hague, R., (1995). Estimation of aminoacids of sericin protein in different developmental stages of multi and bivoltine silkworm races of *Bombyx mori*. *L*. Bull. Seric. Res. **6**: 47-51

Jacob, F., and J. Monod., (1961). Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. 3: 318-356.

Kanda, T and Tamura, T., Microinjection system into the early embryo of the silk worm *Bombyx mori* by using air pressure. Bulletin of The National Institute of Sericultural and Entomological Science, **2**: 32-46.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., (1951). Protein measurement using folin phenol reagent. J. Biol. Chem. 193, 265-275.

Mang, A., Julien, E., Prudhomme, J.C., and Couble, P.A., (1991). Strong inhibitory element down regulates SRE-stimulated transcription of the A3 cytoplasmic action gene *of Bombyx mori*. J. Mol. Biol. **265**: 266-276.

Maniatis, T., and A. E. Fstratiadis (1978). The isolation of structural genes from libraries of Eucaryotic DNA, Cell 15: 687 – 701.

Matsuno, K., Takiyas. (1990). Trancriptional stimulation via SC site of *Bombyx mori* sericin-1 gene through an interaction with a DNA binding protein 5GF-3 Nucleic acid. Res**. 8** (7): 1853-1858.

Mc Carty, M., (1985). The Transforming principle. Discovering that genes are made of Dna. New York Norton.

Mikailov, V.S., Ataeva, D.O., Bronstein, I.B., (1991). Synthesis on double standard circular DNA catalysed by the DNA polymerase alpha DNA primase complex from silkworm cells. Mol. Biol. **25** (2) P+2: 441.

Mounier, N., and Prudhomme, J.C., (1991). Differential expression of muscle and cytoplasmic actin genes during development of *Bombyx mori*. Insect Biochem. **21**, 523-533

Nagaraju, Javare Gowda (1995). DNA finger printing in silkworm *Bombyx mori* using banded krait minor satellite DNA-derived probe. Electrophoresis **16** (6): 1639-1642.

Niranjankumari, S., and Gopinathan, K.P., (1994). DNA polymerase α-primase complex from the silkglands of non-mulberry silkworm *philosuimia ricini*. Biochem. J. **298** (3): 529-535.

Ohta, T., Hirose, S. (1990). Purification of a DNA supercoiling factor from the posterior silk gland of *Bombyx mori* Proc. Natl. Acad. Sci. USA. **87** (14): 5307-5311.

Ouyang, C., Sprague, K.U., (1998). Cloning and characterization of the TATA-binding protein of the silkworm *Bombyx mori*. Gene. 23, **221** (3): 207-213.

Quan, Guo-Zing, Eiji Kanke and Hideki Kawasaki., (1998). Isolation & Particular expression of a new β-rubulin gene in wing discs during metamorphosis of *Bombyx mori*. J. Seric. Science of Japan **67** (1) Feb: 43-50.

Radhakrishna Rao, C., Mitra, S.K., Mathai, A., and Ramamurthy, K.G. Formulae and tables for statistical works 2nd edition Statistical Publishing Society, Calcutta, India.

Robertson, H.M., and Asplund, M.L., (1996). Bmmarl: a basal lineage of the mariner family of transposable elements in the silkworm moth *Bombyx mori*. Insect biochem. Mol. Biol. **26**: 945-954.

Romenenko, E.B., Demindenko, Z.N., (1998). RNA. Polymerase, DNA-polymerase, DNA-methyl transferase and sphingomyllinase activities in liver nuclei of rats of different age. Biochemistry (Moscow) Tech, 159-163.

Sasaki, T., and Hishida, T., (1993). Amino acid sequence of alkaliphilic sericine protease from silkworms. *Bombyx mori* larval digestive juice Febs. Letter, **320** (1): 35-37.

Schneider, W.C., (1957). Determination of nucleic acid in tissues by pentose analysis. In methods in Enzymology (Eds Colovick, S.P., and Kapalan, N.O.) vol.III Academic Press, New York.

Shankernarayanan, P., Banerjee., M., Kacker., (1997). Electrophoresis 18, 1693-1700.

Southern, E.M., (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**, 503-17.

Taira, H. and Kamiek and Kakuta, A., (1993). Nucleotide sequence of the (*Bombyx mori*) cDNA encoding silk gland elongation factor iB nucleic acid Res. **20** (24): 6734.

Tamura Toshiki, Thibert Chantal, (Jan-2000). Germline transformation of the silkworm *Bombyx mori L.* using a piggy Bac transposon-derived vector nature Biotechnology vol **18**: 81-84.

Thomas, G., and D.A. Wilson (1973). Cyclodromes and plaindromes in chromosomes. Cold spring Harbor Symp. Quant. Biol. 38: 353-370.

Ueda, Satoru, (1982). Theory of the growth of Silkworm larvae and its application. J. Agri. Res. Quarterly, 15(3).

Ueda,H,Hirose,S,(1990).Identification and purification of *Bombyx mori* homologue ofFTZ-F1.Nucleic acid res.**18,**7229-7234.

Xinchang, Yawen Qian. (1999). Purification, characterization and cDNA cloning of a new fibrinogenlytic venom protein, Agkisgutacin from Agkistrodon acutus venom Biochem and Biophys. Research Comm. **265**: 530-535. .

Yamao, M., and Ejiris., (1999). Gene targeting in the silkworm by use of a baculovirus. Genes Dev. **13**: 511-516.