OOGENESIS IN *RHODNIUS PROLIXUS*:

A SEARCH FOR *DROSOPHILA* MATERNAL GENE HOMOLOGIES

BY

QUN LI

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN THE PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

UNIVERSITY OF MANITOBA

JULY, 1993

ISBN 0-315-85922-9

Canada

Name _____

Dissertation Abstracts International is arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation. Enter the corresponding four-digit code in the spaces provided.

M.Sc. Zoology

SUBJECT TERM

0472 U·M·I

SUBJECT CODE

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS

Architecture 0729
Art History 0377
Cinema 0900
Dance 0378
Fine Arts 0357
Information Science 0723
Journalism 0391
Library Science 0399
Mass Communications 0708
Music 0413
Speech Communication 0459
Theater 0465

EDUCATION

General 0515
Administration 0514
Adult and Continuing 0516
Agricultural 0517
Art 0273
Bilingual and Multicultural 0282
Business 0688
Community College 0275
Curriculum and Instruction 0727
Early Childhood 0518
Elementary 0524
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998
Physical 0523
Psychology 0525
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS

Language
- General 0679
- Ancient 0289
- Linguistics 0290
- Modern 0291

Literature
- General 0401
- Classical 0294
- Comparative 0295
- Medieval 0297
- Modern 0298
- African 0316
- American 0591
- Asian 0305
- Canadian (English) 0352
- Canadian (French) 0355
- English 0593
- Germanic 0311
- Latin American 0312
- Middle Eastern 0315
- Romance 0313
- Slavic and East European 0314

PHILOSOPHY, RELIGION AND THEOLOGY

Philosophy 0422
Religion
- General 0318
- Biblical Studies 0321
- Clergy 0319
- History of 0320
- Philosophy of 0322

Theology 0469

SOCIAL SCIENCES

American Studies 0323
Anthropology
- Archaeology 0324
- Cultural 0326
- Physical 0327

Business Administration
- General 0310
- Accounting 0272
- Banking 0770
- Management 0454
- Marketing 0338

Canadian Studies 0385
Economics
- General 0501
- Agricultural 0503
- Commerce-Business 0505
- Finance 0508
- History 0509
- Labor 0510
- Theory 0511

Folklore 0358
Geography 0366
Gerontology 0351
History
- General 0578
- Ancient 0579
- Medieval 0581
- Modern 0582
- Black 0328
- African 0331
- Asia, Australia and Oceania 0332
- Canadian 0334
- European 0335
- Latin American 0336
- Middle Eastern 0333
- United States 0337

History of Science 0585
Law 0398
Political Science
- General 0615
- International Law and Relations 0616
- Public Administration 0617

Recreation 0814
Social Work 0452
Sociology
- General 0626
- Criminology and Penology 0627
- Demography 0938
- Ethnic and Racial Studies 0631
- Individual and Family Studies 0628
- Industrial and Labor Relations 0629
- Public and Social Welfare 0630
- Social Structure and Development 0700
- Theory and Methods 0344

Transportation 0709
Urban and Regional Planning 0999
Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES

Agriculture
- General 0473
- Agronomy 0285
- Animal Culture and Nutrition 0475
- Animal Pathology 0476
- Food Science and Technology 0359
- Forestry and Wildlife 0478
- Plant Culture 0479
- Plant Pathology 0480
- Plant Physiology 0817
- Range Management 0777
- Wood Technology 0746

Biology
- General 0306
- Anatomy 0287
- Biostatistics 0308
- Botany 0309
- Cell 0379
- Ecology 0329
- Entomology 0353
- Genetics 0369
- Limnology 0793
- Microbiology 0410
- Molecular 0307
- Neuroscience 0317
- Oceanography 0416
- Physiology 0433
- Radiation 0821
- Veterinary Science 0778
- Zoology 0472

Biophysics
- General 0786
- Medical 0760

EARTH SCIENCES

Biogeochemistry 0425
Geochemistry 0996
Geodesy 0370
Geology 0372
Geophysics 0373
Hydrology 0388
Mineralogy 0411
Paleobotany 0345
Paleoecology 0426
Paleontology 0418
Paleozoology 0985
Palynology 0427
Physical Geography 0368
Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES

Environmental Sciences 0768
Health Sciences
- General 0566
- Audiology 0300
- Chemotherapy 0992
- Dentistry 0567
- Education 0350
- Hospital Management 0769
- Human Development 0758
- Immunology 0982
- Medicine and Surgery 0564
- Mental Health 0347
- Nursing 0569
- Nutrition 0570
- Obstetrics and Gynecology 0380
- Occupational Health and Therapy 0354
- Ophthalmology 0381
- Pathology 0571
- Pharmacology 0419
- Pharmacy 0572
- Physical Therapy 0382
- Public Health 0573
- Radiology 0574
- Recreation 0575
- Speech Pathology 0460
- Toxicology 0383

Home Economics 0386

PHYSICAL SCIENCES

Pure Sciences

Chemistry
- General 0485
- Agricultural 0749
- Analytical 0486
- Biochemistry 0487
- Inorganic 0488
- Nuclear 0738
- Organic 0490
- Pharmaceutical 0491
- Physical 0494
- Polymer 0495
- Radiation 0754

Mathematics 0405
Physics
- General 0605
- Acoustics 0986
- Astronomy and Astrophysics 0606
- Atmospheric Science 0608
- Atomic 0748
- Electronics and Electricity 0607
- Elementary Particles and High Energy 0798
- Fluid and Plasma 0759
- Molecular 0609
- Nuclear 0610
- Optics 0752
- Radiation 0756
- Solid State 0611

Statistics 0463

Applied Sciences

Applied Mechanics 0346
Computer Science 0984
Engineering
- General 0537
- Aerospace 0538
- Agricultural 0539
- Automotive 0540
- Biomedical 0541
- Chemical 0542
- Civil 0543
- Electronics and Electrical 0544
- Heat and Thermodynamics 0348
- Hydraulic 0545
- Industrial 0546
- Marine 0547
- Materials Science 0794
- Mechanical 0548
- Metallurgy 0743
- Mining 0551
- Nuclear 0552
- Packaging 0549
- Petroleum 0765
- Sanitary and Municipal 0554
- System Science 0790

Geotechnology 0428
Operations Research 0796
Plastics Technology 0795
Textile Technology 0994

PSYCHOLOGY

General 0621
Behavioral 0384
Clinical 0622
Developmental 0620
Experimental 0623
Industrial 0624
Personality 0625
Physiological 0989
Psychobiology 0349
Psychometrics 0632
Social 0451

Nom _____

Dissertation Abstracts International est organisé en catégories de sujets. Veuillez s.v.p. choisir le sujet qui décrit le mieux votre thèse et inscrivez le code numérique approprié dans l'espace réservé ci-dessous.

SUJET

CODE DE SUJET

U·M·I

Catégories par sujets

HUMANITÉS ET SCIENCES SOCIALES

COMMUNICATIONS ET LES ARTS

Architecture 0729
Beaux-arts 0357
Bibliothéconomie 0399
Cinéma 0900
Communication verbale 0459
Communications 0708
Danse 0378
Histoire de l'art 0377
Journalisme 0391
Musique 0413
Sciences de l'information 0723
Théâtre 0465

ÉDUCATION

Généralités 515
Administration 0514
Art 0273
Collèges communautaires 0275
Commerce 0688
Économie domestique 0278
Éducation permanente 0516
Éducation préscolaire 0518
Éducation sanitaire 0680
Enseignement agricole 0517
Enseignement bilingue et multiculturel 0282
Enseignement industriel 0521
Enseignement primaire. 0524
Enseignement professionnel 0747
Enseignement religieux 0527
Enseignement secondaire 0533
Enseignement spécial 0529
Enseignement supérieur 0745
Évaluation 0288
Finances 0277
Formation des enseignants 0530
Histoire de l'éducation 0520
Langues et littérature 0279
Lecture 0535
Mathématiques 0280
Musique 0522
Orientation et consultation 0519
Philosophie de l'éducation 0998
Physique 0523
Programmes d'études et enseignement 0727
Psychologie 0525
Sciences 0714
Sciences sociales 0534
Sociologie de l'éducation 0340
Technologie 0710

LANGUE, LITTÉRATURE ET LINGUISTIQUE

Langues
- Généralités 0679
- Anciennes 0289
- Linguistique 0290
- Modernes 0291

Littérature
- Généralités 0401
- Anciennes 0294
- Comparée 0295
- Médiévale 0297
- Moderne 0298
- Africaine 0316
- Américaine 0591
- Anglaise 0593
- Asiatique 0305
- Canadienne (Anglaise) 0352
- Canadienne (Française) 0355
- Germanique 0311
- Latino-américaine 0312
- Moyen-orientale 0315
- Romane 0313
- Slave et est-européenne 0314

PHILOSOPHIE, RELIGION ET THÉOLOGIE

Philosophie 0422
Religion
- Généralités 0318
- Clergé 0319
- Études bibliques 0321
- Histoire des religions 0320
- Philosophie de la religion 0322

Théologie 0469

SCIENCES SOCIALES

Anthropologie
- Archéologie 0324
- Culturelle 0326
- Physique 0327

Droit 0398
Économie
- Généralités 0501
- Commerce-Affaires 0505
- Économie agricole 0503
- Économie du travail 0510
- Finances 0508
- Histoire 0509
- Théorie 0511

Études américaines 0323
Études canadiennes 0385
Études féministes 0453
Folklore 0358
Géographie 0366
Gérontologie 0351
Gestion des affaires
- Généralités 0310
- Administration 0454
- Banques 0770
- Comptabilité 0272
- Marketing 0338

Histoire
- Histoire générale 0578
- Ancienne 0579
- Médiévale 0581
- Moderne 0582
- Histoire des noirs 0328
- Africaine 0331
- Canadienne 0334
- États-Unis 0337
- Européenne 0335
- Moyen-orientale 0333
- Latino-américaine 0336
- Asie, Australie et Océanie 0332

Histoire des sciences 0585
Loisirs 0814
Planification urbaine et régionale 0999
Science politique
- Généralités 0615
- Administration publique 0617
- Droit et relations internationales 0616

Sociologie
- Généralités 0626
- Aide et bien-âtre social 0630
- Criminologie et établissements pénitentiaires 0627
- Démographie 0938
- Études de l' individu et de la famille 0628
- Études des relations interethniques et des relations raciales 0631
- Structure et développement social 0700
- Théorie et méthodes. 0344
- Travail et relations industrielles 0629

Transports 0709
Travail social 0452

SCIENCES ET INGÉNIERIE

SCIENCES BIOLOGIQUES

Agriculture
- Généralités 0473
- Agronomie. 0285
- Alimentation et technologie alimentaire 0359
- Culture 0479
- Élevage et alimentation 0475
- Exploitation des pâturages 0777
- Pathologie animale 0476
- Pathologie végétale 0480
- Physiologie végétale 0817
- Sylviculture et faune 0478
- Technologie du bois 0746

Biologie
- Généralités 0306
- Anatomie 0287
- Biologie (Statistiques) 0308
- Biologie moléculaire 0307
- Botanique 0309
- Cellule 0379
- Écologie 0329
- Entomologie 0353
- Génétique 0369
- Limnologie 0793
- Microbiologie 0410
- Neurologie 0317
- Océanographie 0416
- Physiologie 0433
- Radiation 0821
- Science vétérinaire 0778
- Zoologie 0472

Biophysique
- Généralités 0786
- Medicale 0760

SCIENCES DE LA TERRE

Biogéochimie 0425
Géochimie 0996
Géodésie 0370
Géographie physique 0368
Géologie 0372
Géophysique 0373
Hydrologie 0388
Minéralogie 0411
Océanographie physique 0415
Paléobotanique 0345
Paléoécologie 0426
Paléontologie 0418
Paléozoologie 0985
Palynologie 0427

SCIENCES DE LA SANTÉ ET DE L'ENVIRONNEMENT

Économie domestique 0386
Sciences de l'environnement 0768
Sciences de la santé
- Généralités 0566
- Administration des hipitaux 0769
- Alimentation et nutrition 0570
- Audiologie 0300
- Chimiothérapie 0992
- Dentisterie 0567
- Développement humain 0758
- Enseignement 0350
- Immunologie 0982
- Loisirs 0575
- Médecine du travail et thérapie 0354
- Médecine et chirurgie 0564
- Obstétrique et gynécologie 0380
- Ophtalmologie 0381
- Orthophonie 0460
- Pathologie 0571
- Pharmacie 0572
- Pharmacologie 0419
- Physiothérapie 0382
- Radiologie 0574
- Santé mentale 0347
- Santé publique 0573
- Soins infirmiers 0569
- Toxicologie 0383

SCIENCES PHYSIQUES

Sciences Pures

Chimie
- Généralités 0485
- Biochimie 487
- Chimie agricole 0749
- Chimie analytique 0486
- Chimie minérale 0488
- Chimie nucléaire 0738
- Chimie organique 0490
- Chimie pharmaceutique 0491
- Physique 0494
- PolymÇres 0495
- Radiation 0754

Mathématiques 0405
Physique
- Généralités 0605
- Acoustique 0986
- Astronomie et astrophysique 0606
- Électronique et électricité 0607
- Fluides et plasma 0759
- Météorologie 0608
- Optique 0752
- Particules (Physique nucléaire) 0798
- Physique atomique 0748
- Physique de l'état solide 0611
- Physique moléculaire 0609
- Physique nucléaire 0610
- Radiation 0756

Statistiques 0463

Sciences Appliqués Et Technologie

Informatique 0984
Ingénierie
- Généralités 0537
- Agricole 0539
- Automobile 0540
- Biomédicale 0541
- Chaleur et ther modynamique 0348
- Conditionnement (Emballage) 0549
- Génie aérospatial 0538
- Génie chimique 0542
- Génie civil 0543
- Génie électronique et électrique 0544
- Génie industriel 0546
- Génie mécanique 0548
- Génie nucléaire 0552
- Ingénierie des systâmes 0790
- Mécanique navale 0547
- Métallurgie 0743
- Science des matériaux 0794
- Technique du pétrole 0765
- Technique minière 0551
- Techniques sanitaires et municipales 0554
- Technologie hydraulique 0545

Mécanique appliquée 0346
Géotechnologie 0428
Matières plastiques (Technologie) 0795
Recherche opérationnelle 0796
Textiles et tissus (Technologie) 0794

PSYCHOLOGIE

Généralités 0621
Personnalité 0625
Psychobiologie 0349
Psychologie clinique 0622
Psychologie du comportement 0384
Psychologie du développement 0620
Psychologie expérimentale 0623
Psychologie industrielle 0624
Psychologie physiologique 0989
Psychologie sociale 0451
Psychométrie 0632

OOGENESIS IN *RHODNIUS PROLIXUS*: A SEARCH FOR *DROSOPHILA* MATERNAL GENE HOMOLGIES

BY

QUN LI

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

ABSTRACT

Insights have been gained into the mechanisms of the establishment of embryonic axis by the actions of maternal genes in *Drosophila melanogaster*. The degree of conservation of these maternal genes in other organisms is of significant interest. I screened *Rhodnius* for homology with the *Drosophila* maternal genes *Bic-D, nanos, vasa, bicoid*. The presence of these genes in *Rhodnius* genome and expression in *Rhodnius* ovarioles was studied using Southern blot and Northern blot hybridization using the cDNAs of these *Drosophila* genes as probes.

No hybridization with *Bic-D* and *nanos* was detected in the *Rhodnius* genome. Eight hybridization bands were found on Southern blots of *Rhodnius* genomic DNA and two weak hybridization bands (sized 1.8 Kb and 0.3Kb) were found on *Rhodnius* ovariole RNA blots, when probed with *vasa* cDNA. Two strong hybridization bands (sized 4.6 Kb and 3.2 Kb) on *Rhodnius* genomic DNA Southern blots, two hybridization bands (sized 2.8 Kb and 0.53 Kb) on *Rhodnius* ovariole RNA blots were detected when probed with *bicoid* cDNA.

The homology of *Rhodnius* DNA to *bicoid* gene was further analysed by probing the Southern blots and Northern blots individually with the several restriction-enzyme-digested DNA fragments of the *bicoid* cDNA. Only a 252 bp fragment containing a PRD-repeat of the *bicoid* cDNA was responsible for the hybridization on both the Southern blots and the Northern blots. This fragment contains a 90 bp DNA motif encoding for a repetitive histidine-proline sequence.

A synthetic oligonucleotide of the PRD-repeat hybridized to the same two

4.6 Kb and 3.2 Kb bands on Southern blots detected when probed with *bicoid* full length cDNA. There was no hybridization detected on the Northern blots.

My research showed the conservation of the PRD-repeat and possibly part of the other sequence of the *bicoid* gene in the *Rhodnius* genome. The expression of this other sequence in the *Rhodnius* ovarioles is within the 252 bp fragment of *bicoid* cDNA. This is the first evidence of a PRD-repeat found outside of *Drosophila*.

ACKNOWLEDGEMENTS

To begin, I would like to thank Dr. Erwin Huebner, my advisor, for having me in the Lab, for his confidence to me in letting me do molecular biology, for his encouragement to me for each progress I made, also for his countless help in finishing my thesis. Doc, I do not know how to express my appreciation to your advice, but all I can say is "Thank you, my Shi-fu (master)!". I will hold the "red thread of science" for sure.

I would like to thank Dr. Ross McGowan, a true gentleman and scientist, for showing me the rope to do molecular biology, for his inspiring discussion, for letting me use his equipment, also for his kindness and fairness to me. Ross, I felt great to work with you, your scientific attitude will be my example in the future.

I also thank Dr.G.R.Klassen, for his critical comments which improved this thesis immeasurably.

I sincerely thank Bill and Charlene Diehl-Jones for their friendship and kindness to me. The good time we had at Spruce Woods will never be forgotten. I also thank Bill for providing experiment protocol and *vasa* gene cDNA.

I thank Cris, Dr.T.Wiens, Shiuwei, Mingchuan, all my lab partners for providing help and friendship.

I thank my brother-in-law, Dr.Linruo Zhao, and my sister Qing, for introducing me to Dr.E.Huebner's Lab, and for their financial help.

The last,and most important person I would like to thank is my wife, Zhao-hui. Her understanding and spiritual support sustained me during these years.

TABLE OF CONTENTS

INTRODUCTION

Early in embryogenesis, cells may acquire specific developmental fates with the consequence that their descendants will express characteristic sets of genes and a body pattern or polarity has already been determined. A central question in developmental biology is how cells in different regions of an embryo acquire different developmental fates and how the polarity of an embryo is determined.

Wilson (1928) proposed that maternal factors are differentially distributed in the egg and become localized in specific groups of cells by cleavage thereby specifying their fate. Maternal factors, or cytoplasmic determinants have been implicated in the differentiation of ciliated epithelia (Chung and Malasinski,1983; Kageura and Yamana,1984), embryonic muscle (Gurdon *et al.*,1985) and germ cells (Smith,1966; Buehr and Blackler,1970) in frog embryos. Localized maternal components, either in a discrete or in a graded fashion, are involved in establishing the dorsal-ventral axis of *Xenopus* embryo (Nieuwkoop,1977; Gerhart *et al.*,1983) and in determining the anterior-posterior polarity (Nusslein-Volhard,*et al.*,1987) and also the formation of the germ cells of *Drosophila* (Okada *et al.*,1974; Lehmann and Nusslein-Volhard,1986).

It appears that maternal mRNAs and/or proteins asymmetrically localized in the egg cortex act as cytoplasmic determinants. Studies utilising *in situ* hybridization, molecular cloning and analytical microscopy have revealed that maternal mRNA's exhibit unique spatial distributions in the developing oocytes, eggs or embryos of sea urchins (Rogers and Gross,1978; Venezsky *et al.*, 1981;

Showman *et al.*, 1982; Moon *et al.*, 1983), ascidians (Jeffery *et al.*, 1978, 1983, 1984), *Chaetopterus* (Jeffery and Wilson, 1983; Jeffery, 1985), *Xenopus laevis* (Capco and Jeffery, 1982; Carpenter and Klein, 1982; Phillips, 1982; Yisraeli,*et al.*, 1989), *Drosophila* (see review of Nusslein-Volhard, 1991; St Johnson and Nusslein-Volhard, 1992) and silkworm (Paglia,*et al.*, 1976; Kastern,*et al.*, 1990). Localization of maternal mRNAs and/proteins have been best studied in *Xenopus*, *Drosophila* and various ascidians.

In *Xenopus laevis*, a small class of mRNAs that are unevenly distributed in oocytes and eggs have been identified (Rebagliati *et al.*,1985; King and Barklis, 1985). The mRNA of the Vg1 gene was found to be localized in the vegetal cortex of the oocyte during oogenesis (Melton, 1987; Yisraeli and Melton, 1988). Vg1 codes a protein which is a member of the transforming growth factor-β family (TGF-β) (Weeks and Melton, 1987). The role of Vg1 in *Xenopus* early development is not clear. Recently, another maternal gene called Xcat-2 was also found to be localized in the vegetal cortical region, and encodes a protein that belongs to the CCHC RNA-binding family of zinc finger proteins and shares a homologous region with one *Drosophila* maternal gene *nanos* (Mosquera *et al.*,1993).

Research on the establishment of pattern and polarity in the *Drosophila* embryo highlights insect oogenesis and embryogenesis as a powerful developmental model. Insight into the mechanisms of the establishment of embryonic anterior-posterior and dorsal-ventral axes of *Drosophila* embryos have emerged, and have had significant implications in the analysis of other

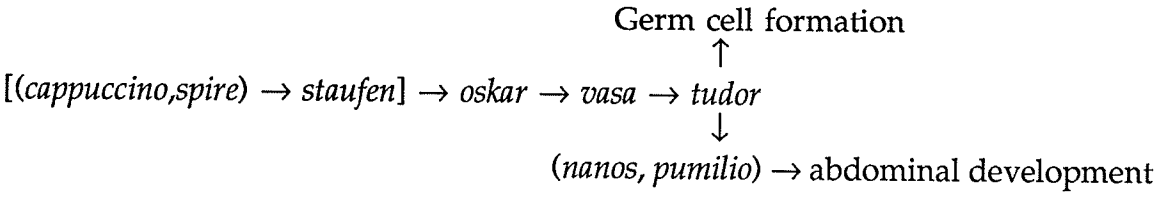
invertebrate and vertebrate systems. The axes are controlled by four localized maternal signals which are the anterior system, the posterior system, the terminal system and the dorsal-ventral system (St Johnston and Nusslein-Volhard,1992).

The anterior system is the simplest and best studied of the four systems at the present time. The *bicoid* gene is indispensable for the determination of all the anterior structures including the head and thorax, while mutations in the other genes of the system have only partial effects on the anterior pattern (Frohnhofer and Nusslein-Volhard,1986,1987). The *bicoid* mRNA is synthesized in the nurse cells during oogenesis and translocated to the anterior pole of the egg during oogenesis. It remains localized until fertilization when the *bicoid* mRNA is translated. This produces an anterior to posterior concentration gradient of *bicoid* protein that extends over the anterior two-thirds of the embryo (Frigerio *et al.*, 1986; Berleth *et al.*, 1988; Driever and Nusslein-Volhard, 1988a; St Johnston *et al.*, 1989). The *bicoid* protein gradient is sufficient to determine the polarity and pattern of the anterior half of the embryo. As the number of copies of *bicoid* is increased, more RNA and protein is produced, resulting in an expansion of the *bicoid* protein gradient toward the posterior. This change in the extent of the gradient produces a corresponding change in the position of the head fold at gastrulation (Driever and Nusslein-Volhard, 1989b; Struhl *et al.*, 1990). Injection of *in vitro* synthesized *bicoid* RNA into other positions in the embryo results in a protein gradient that directs the formation of ectopic head and thoracic structures, with the most anterior pattern elements forming closest to the site of injection.

The basic pathway through which *bicoid* gene determines the anterior structure is that *bicoid* protein regulates the expression of one set of genes called gap genes. Gap gene's products regulate the activity of the pair rule genes (see references in Zhang,*et al.*, 1991), thereby ensuring the correct establishment of segment primordia within their functional domains. One target of the *bicoid* protein is the gap gene *hunchback* which is first transcribed at the syncytial blastoderm stage in a large anterior domain extending about 50% of the egg length and is required for the development of thorax and part of head (Lehmann and Nusslein-Volhard,1987a; Tautz,*et al.*, 1987). *Bicoid* protein contains a homeodomain and is sufficient to directly activate the zygotic expression of the *hunchback* gene (Driever and Nusslein-Volhard, 1989a, 1989b; Schroder *et al.*, 1988; Struhl *et al.*, 1989).

The posterior pole plasm contains the signals that determine the formation of germ cells and the development of the abdomen. A cascade of genes is involved in the determination of the posterior structure. Figure 1 summaries the sequence of the action of these genes.

Figure 1.



Spire and *cappuccino* are required for *staufen* protein localization (St Johnston *et al.*, 1991), *cappuccino,spire* and *staufen* are necessary for *oskar* mRNA

localization at the posterior pole of the oocyte during oogenesis (Ephrussi *et al.*, 1991; Kim-Ha *et al.*, 1991). Mislocalization of *oskar* mRNA to the anterior pole of *Drosophila* embryos leads to induction of a certain number of germ cells and abdominal development at the anterior end, indicating that *oskar* plays a key role in directing pole plasm assembly and controlling the number of primordial germ cells normally formed at the posterior pole of the *Drosophila* embryo (Ephrussi and Lehmann,1992). Posteriorly-localized *vasa* protein, along with the *tudor* gene, are indispensable in determination of germ cell formation and abdominal development (Ephrussi and Lehmann,1992). *Vasa* protein has a high degree of homology with members of the helicase class of RNA-binding proteins (Hay *et al.*,1990; Lasko and Ashburner,1990). *Nanos* mRNA is also localized at the posterior end of the egg (Wang and Lehmann,1991). *Nanos* and *pumilio* are specifically involved in the determination of abdomen but not the formation of germ cells (Lehmann and Nusslein-Volhard,1987b,1991). The *nanos* gene, presumably via *nanos* protein, prevents the translation of the uniformly distributed *hunchback* mRNA only at the posterior end (Wharton and Struhl,1991), instead of directly activating other gene expression. Besides directly determining the anterior structure, *hunchback* protein also acts as a transcriptional repressor of two gap genes *knirps* and *giant* which are both required for the formation of the abdominal pattern at the posterior end (Mohler *et al.* 1989; Pankratz *et al.* 1989; Hulskamp *et al.* 1989; Struhl,1989). Thus, *nanos* successfully blocks the invasion of anterior gene products (like *hunchback*) to the posterior region so only the genes

responsible for the posterior development are expressed in the posterior region. In the absence of maternal *hunchback* mRNA, *nanos* is not required for normal abdominal development.

The terminal system is responsible for the development of anterior-most and posterior-most regions of the embryo, the acron and telson.The dorsal-ventral system is responsible for the determination of the dorsal-ventral axis of *Drosophila* (Nusslein-Volhard,1991; St Johnston and Nusslein-Volhard,1992). These two systems are more complicated than, and different from, the anterior and posterior systems. The exact pathway of the terminal and dorsal-ventral determination is still not clear, however, the present working hypothesis is that the egg produces a transmembrane protein that is uniformly distributed in the egg membrane. This protein then acts as a receptor for the ligand that is locally produced by the surrounding follicle cells. The receptor in the egg membrane can be activated by the locally produced ligand and a signal transduction cascade results. This leads to a positive control of transcription of zygotic target genes.

The mechanisms of how the maternal determinants are localized is still not well understood. It has been established that the cytoskeleton is essential for the translocalization of maternal determinants. The translocation of Vg1 to the vegetal hemisphere of *Xenopus* oocyte requires microtubules and the stable anchoring of Vg1 at the vegetal cortex requires cortex microfilaments (Yisraeli, *et al.*,1990). Similarly, in *Drosophila*, the translocalization of *bicoid* mRNA from the nurse cells to the anterior end of the oocyte is also dependent upon microtubules (Pokrywka

and Stephenson, 1991). Theurkauf,*et al.* (1992) found that the reorganization of microtubules along the anterior-posterior axis of *Drosophila* oocyte is concomitant with the localization of anterior determinant *bicoid* mRNA and the posterior determinant *oskar* mRNA. They suggested that the polarized arrangement of microtubules might be involved in the determination of oocyte polarity. Regarding the specific localization of maternal determinants, the studies of *Xenopus* and *Drosophila* system showed that the 3′ untranslated portion of the *bicoid* and Vg1 mRNA are both necessary and sufficient to direct the specific localization of its mRNA suggesting that this specific localization is mediated by a sequence-specific trapping system (Macdonald and Struhl,1988; Macdonald,1990; Mowry and Melton,1992).

Although significant insight has resulted from research on oogenesis and the determination of the embryonic pattern in a few systems like *Xenopus* and *Drosophila*, relatively few other systems have been investigated. How universal is the mechanism of maternal determination ? In particular, the degree of conservation of the genes that play roles in development in other organisms is of significant interest. Since a great deal has yet to be learned about the cell biology of the differential localization of maternal determinants in oocyte, there is a need to explore a diversity of systems besides *Drosophila*. Some may possess a more suitable cellular architecture that may be exploitable to allow us to dissect the pathway of the localization of maternal determinants. The telotrophic ovaries of Hemiptera provide an ideal system to study the formation of polarity in oogenesis

and translocation of nurse cell specific mRNA's in this process. Due to its unique cytoarchitecture it offers significant advantages over the polytrophic system, characteristic of *Drosophila*.

The unique structure of the meroistic telotrophic ovary of *Rhodnius* has been well documented (Huebner,1984a,b.). However, reiteration of the relevant aspects provides a useful background for this study. There are two ovaries within each *Rhodnius* female, each is composed of seven ovarioles. The ovariole is divided into two distinct regions, the tropharium and vitellarium. The tropharium consists of a syncytium of nurse cells connected by intracellular bridges (Huebner,1984). The vitellarium is composed of several developing oocytes which are arrested at meiotic prophase I and the surrounding somatic follicle cells. Unlike the polytrophic *Drosophila* ovarioles, where the nurse cells and the oocyte are closely connected by a ring canal (King,1970), each *Rhodnius* oocyte is spatially separated from and connected to the common tropharium by a long trophic cord (up to 1 mm) which is filled with stable microtubules (Huebner,1984). There is only one oocyte in vitellogenesis per ovariole while the remaining oocytes are in previtellogenesis (Huebner,1984) (see figure 2). It has been shown that the *Rhodnius* nurse cells are highly active in the synthesis of mRNA, rRNA, proteins and mitochondria while the nucleus of the oocyte (germinal vesicle) is inactive especially in RNA synthesis, and these materials

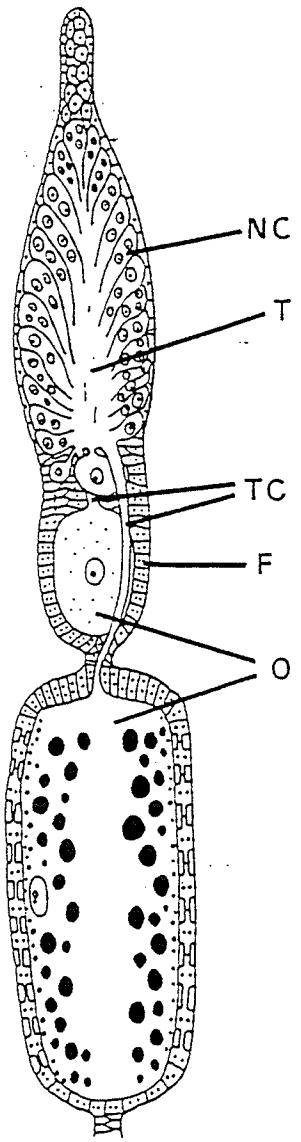


Figure 2. Summary diagram of the adult *Rhodnius* ovariole.

Nurse cell (NC), oocytes (o), trophic core (T), trophic cords (TC), Follicle cells (F). (From Huebner,1984b).

are transported from the nurse cells to the developing oocytes via the trophic cords (Vanderberg, 1963; Huebner, 1984a,b.). Thus if there are any mRNA's that act as maternal determinants during oogenesis of *Rhodnius* oocyte, they are presumably synthesized in the tropharium and transported to the oocytes via the trophic cords. This unique cellular architecture of the *Rhodnius* ovariole provides an excellent model in the future to study the mechanism of intracellular transport of mRNA's. The identification of any maternal mRNA's that play roles in development, especially in polarity and or germ cell determination would open new avenues to explore the exaggerated nurse cell - oocyte polarity in the telotrophic ovarioles. There has been no previous research on the existence of maternal determinants in the *Rhodnius* ovariole system.

Recently, the study of McPherson and Huebner (1993) showed that there are also dynamic and asymmetrical arrangements of cytoskeleton in the anterior (the region close to the trophic cord)- posterior (the region far away from the trophic cord) ends of the developing *Rhodnius* oocyte similar to the *Drosophila* system. This suggests that that cytoskeleton might also play a similar role in determining the asymmetrical localization of the potential maternal determinants in *Rhodnius*. Also, asymmetries are found in the radial pattern of extracellular electrical currents around the intact *Rhodnius* ovarioles and particularly around the terminal follicle (Diehl-Jones and Huebner, 1992). This also implies the existence of developmental polarity in the *Rhodnius* oocyte. Recent study on the origin of germ cells in early embryogenesis of *Rhodnius* also provides direct

evidence of asymmetries and polarity in the *Rhodnius* oocyte (Heming and Huebner, personal communication; Kelly,1989).

Drosophila is the only insect system where the molecules responsible for the maternal determination of the embryonic pattern have been identified at the molecular level (St Johnston and Nusslein-Volhard,1992). This coupled with the availability of the cDNA's of the *Drosophila* maternal genes, which makes it possible for us to attempt to identify the existence the homologous genes in *Rhodnius* using molecular biology tools. My M.Sc. research is the first attempt to determine if the selected maternal genes from *Drosophila* have any homologous counterparts in the insect *Rhodnius prolixus* which is from a different order. This is done by screening the *Rhodnius* genomic DNA Southern blots and ovariole RNA blots with the cDNA's of *Drosophila* maternal genes as probes in order to see if the any of the selected *Drosophila* maternal genes have homologous sequences which are conserved in the *Rhodnius* genome and expressed in the *Rhodnius* ovariole. Detection of any homologies would make it possible to determine if they play polarity or germ cell determining roles in *Rhodnius* and how conserved these mechanisms are. Furthermore, the identification of specific nurse cell produced mRNA's in *Rhodnius* would also provide a valuable tool for the study of the mechanism and dynamics of nurse cell - oocyte transport in the telotrophic ovary and the possible role of cytoskeleton in the establishment of polarities.

Thus the objectives of my research are (1) to isolate genomic DNA from *Rhodnius;* (2) to screen the *Rhodnius* genomic DNA using Southern blot

hybridization with the probes of the *Drosophila* maternal genes *nanos, Bic-D, oskar,bicoid* and *vasa* as probes; (3) to isolate total RNA from *Rhodnius* ovarioles and testis; (4) to detect the tissue expression of the homologous sequences in the *Rhodnius* genome to the *Drosophila* genes through Northern blot hybridization using the probes that gave positive signals on Southern blots; (5). to examine which part of the genes that gave positive signal is responsible for the hybridization.

MATERIALS AND METHODS

Animal Rearing Techniques

A colony of *Rhodnius prolixus* was kept in a controlled environment at high humidity and 27°C according to the methods of Huebner and Anderson (1972). The colony was fed at two week intervals on female New Zealand white rabbits. A colony of wild type *Drosophila melanogaster* was also kept at room temperature in a vial with sponge plug containing Carolina Instant *Drosophila* Media (Carolina Biological Supply Co.).

Animal Dissection

Ovaries and testes were dissected from adult *Rhodnius,* fed four or five days before. The legs, wings, lateral edges of the abdomen were quickly removed with surgical scissors and the anterior dorsal cuticle layer was peeled off. The

ovaries or testes were removed from the surrounding tissues and transferred to a 1.5 ml microfuge tube on ice.

Isolation of *Rhodnius* Genomic DNA.

The following method (protocol of Dr.Ross McGowan, Dept. of Zoology, UM) was used to isolate *Rhodnius* genomic DNA. For each isolation, ovarioles from 8 *Rhodnius* females were placed into an ice-cold 1.5 ml microfuge tube to which 600 ul "tail buffer" (Tris.pH 8.0: 50 mM, EDTA pH 8.0: 100 mM, NaCl:100 mM, SDS:1%) was added. The ovarioles were homogenized with a pellet pestle (Mandel Sci. Co. Ltd), 50 ug proteinase K (stock 10 mg/ml, Promega) was added to the homogenate followed by overnight incubation at 56°C. Then 600 ul (or equal volume) of phenol previously equilibrated with TE^{-4} (Tris,pH 8.0:10 mM, EDTA:0.1 mM) was added to the proteinase K digests and mixed by vortexing, spun at 12,000 g for 10 minutes, and the aqueous phase and interphase were transferred with a broad-tip plastic pipette (Canlab) into another sterile 1.5 ml microfuge tube. Back extraction was done once by adding 100 ul TE^{-4} to the original tube, vortexing and spinning as before and the aqueous phase was recovered and combined with the first aqueous phase and interphase extraction and then equal volume of phenol was added, vortexing and spinning again as before, the aqueous phase and interphase were collected as before into a new 1.5 ml microfuge tube. To this tube, an equal volume of PCI (25 phenol : 24 chloroform : 1 isoamyl alcohol) solution was added, vortexing and spinning as

before for 10 minutes. Only the aqueous phase was transferred to another tube to which 3 ul boiled RNAase A (10 mg/ml stock, Pharmacia) was added and incubated at 37°C for 1 hour. The PCI extraction was repeated once more and the aqueous phase was collected. An equal volume of CIA (24 chloroform : 1 isoamyl alcohol) was added, vortexed and spun for 3 min and the aqueous phase was transferred to another new 1.5 ml microfuge tube. 1/2 volume of 7.5 M ammonium acetate was added to the tube, mixed well, and then an equal volume of isopropanol was added. The tube was inverted several times. The DNA was pelleted by centrifuging at 12,000 g for 10 minutes, and the DNA pellet was washed with 70% and 95% ethanol (the tube was spun between the two washes) and dried in a 65°C oven for 3 - 4 minutes and dissolved in 400 ul of TE^{-4} in 65°C oven for 45 - 60 minutes. The DNA was reprecipitated with ammonium acetate and washed as before. The DNA was redissolved in 400 ul of TE^{-4}. 10 ul of the DNA solution was added to 390 ul TE^{-4} and the concentration was measured at 260 nm and 280 nm on a spectrophotometer (Spectronic®601, Milton Roy Co.). The ratio of the $OD_{260nm/280nm}$ was around 1.8 ~ 2.0. Since 1 unit of the OD value is equal to 50 ug/ml of double stranded DNA, the calculation of the quantity of total DNA in 400 ul solution was: OD value X 50 X 0.4 X 40 ug. The yield of each standard extraction was 60 ~ 80 ug. Usually, 200 ng of DNA was run in a 1% agarose check gel and stained in 0.5 ug/ml ethidium bromide water solution for 15 minutes and viewed under UV. The DNA appeared as a single tight band if it was not degraded.

Isolation of *Drosophila* Genomic DNA

To use *Drosophila melanogaster* genomic DNA as a positive control for Southern blot hybridization, *Drosophila* genomic DNA was also isolated according to a method provided by Dr.Paul Lasko's Lab, Dept.of Biology, McGill Univ.,Montreal. Forty etherized flies were homogenized in 600 ul of "solution A" (Tris-HCl,pH 9.0:0.1 M, EDTA:0.1M, SDS:1%, DEPC(Diethyl pyrocarbonate):1% added freshly) in an ice cold 1.5 ml microfuge tube with pellet pestle. The homogenate was incubated for 30 minutes at 70°C. Then PCI extraction was done twice and CIA extraction once. Three ul of boiled RNAase A (10 mg/ml stock, Pharmacia) was added, incubated at 37°C for 1 hour. Then the PCI and CIA extraction was repeated. Fourteen ul of 8 M potassium acetate was added for each 100 ul homogenate and left on ice for 30 minutes and then spun at 12000 g for 15 minutes at 4°C. The supernatant was transferred to another tube and the DNA was precipitated by adding > 1 volume of isopropanol at room temperature for 20 minutes and spinning for 5 minutes at room temperature. The DNA pellet was washed and dried as usual and then redissolved in 50 ul TE^{-4}. The concentration and quality of the DNA was checked as noted earlier.

DNA Gel Electrophoresis.

1% agarose DNA gel electrophoresis was routinely performed throughout this research. Agarose powder (Sigma A-9539) was weighed and dissolved in 1 X TAE running buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 7.8) by heating in

microwave for 3 minutes and cooled down to 56°C in waterbath. The dissolved agarose was stirred on a stirring plate for one minute and then was poured into a tray containing a comb and solidified over 15 - 30 minutes. DNA sample (50 ~ 1000 ng) was mixed with 1 X DNA loading buffer (20% ficoll, 50 mM EDTA, 10 mM Tris. pH 8.0, 0.25% bromophenol blue, 0.25% xylene cyanole FF) and carefully loaded into the loading well with a micropipitte and run in presence of 1 X TAE running buffer at 80 - 100 volts with a power supply (Bio/Rad Model 250/2.5). Usually a λ bacteriophage genomic HindIII digest (100 ng/ul) was also run as a marker in another lane as well. After the electrophoresis , the gel was stained in an aqueous 0.5 ug/ml ethidium bromide solution for 15 minutes and destained in deionized water for 15 minutes. The DNA was viewed on a UV transluminator (Foto/PrepI BIO/CAN SCIENTIFIC) and photographs were taken with a polaroid camera (Fotodyne®, BIO/CAN SCIENTIFIC Cat № 5-5335).

RNA Isolation.

Total RNA was isolated from *Rhodnius* ovarioles, testis and *Drosophila melanogaster* adult females with the RNAgents™ Total RNA Isolation Kit (Promega). *Rhodnius* ovarioles from 3 ~ 5 females, *Rhodnius* testis from 10 males, 10 *Drosophila* adult females were put into seperate ice-cold 1.5 ml microfuge tubes. 300 ul of solution A (4 M guanidium thiocyanate, 25 mM sodium citrate,pH 7, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol) was added and the tissue was homogenized completely with a pellet pestle (sterilized by soaking in 100%

ethanol overnight) at room temperature. Another 200 ul of solution A was added to the homogenate and then 50 ul (0.1 volume of solution A) of 2 M sodium acetate (pH 4.0) was added and mixed by inversion. This was followed by adding 600 ul of PCI, 10 seconds vortexing, chilling for 15 minutes on ice and finally centrifugation at 10,000 g for 20 minutes at 4°C. Under acidic conditions, only RNA will specifically partition into the aqueous phase. So the aqueous phase was collected with a sterile plastic transfer pipet (Optional: a back extraction was done by adding 10 ul of 2 M sodium acetate (pH4.0) and 100 ul of solution A to the original tube, vortexing and centrifugation as before and the aqueous phase was collected and combined with that of the first extractions). The RNA was precipitated by mixing an equal volume of isopropanol, and leaving at - 20°C for at least 2 hours or overnight. Centrifugation at 10,000 g for 20 minutes at 4°C produced an RNA pellet which was resuspended in 500 ul of solution A at room temperature by slowly pipetting the solution up and down and vortexing. The RNA was reprecipitated, resuspended and reprecipitated again as before. Thus only RNA was differentially precipitated. The RNA pellet was washed twice with 1 ml ice cold 95% ethanol, with a 10 minute spin between each washing . The RNA pellet was dried by heating the tube in a 65°C oven for 2 minutes. The RNA was resuspended in 500 ul of DEPC-treated dd H_2O by pipetting and vortexing. After the reprecipitation of the RNA by adding 0.1 volume of 2 M sodium acetate (pH 4, DEPC treated), and equal volume of isopropanol, it was put at -20°C for 1 hour. Subsequently it was centrifuged, washed, dried and resuspended as

before. The precipitation step was repeated to remove the guanidium thiocyanate in the sample. 1 or 2 ul of RNA sample was added into TE^{-4} solution yielding a total volume of 400 ul. The OD value was read at 260 um, 280 um and 230 um. Each OD unit at 260 um represents 40 ug of RNA per ml. A ratio of OD_{260}/OD_{280} around 1.8 ~ 2.0 indicates there is no protein contamination, and the ratio of OD_{260}/OD_{230} greater than 2 indicates there is no contamination of guanidium thiocyanate. If there was guanidium thiocyanate contamination, the reprecipitation and resuspension was repeated till the guanidium thiocyanate was eliminated. In each standard extraction, 400 - 600 ug, 100 - 200 ug or 50 - 80 ug of total RNA was isolated from *Rhodnius* ovarioles,*Rhodnius* testis or *Drosophila* female adults, respectively. The integrity of RNA was checked by running a RNA denaturing gel and the RNA was stained with ethidium bromide and viewed with a UV transluminator to visualize distinct 28S, 18S and 5S ribosomal RNA bands. I also used mouse β-actin cDNA to probe the RNA blot to verify the RNA integrity by checking for a discrete actin RNA band . For RNA isolation, all salt solutions and water were treated with DEPC (diethyl pyrocarbonate) to inactivate any RNAase. DEPC was added to the solution to final concentration of 0.05% and shaken vigorously and left overnight at room temperature overnight, and then autoclaved for 20 minutes .

Electrophoresis of RNA in Gels Containing Formaldehyde.

The RNA sample was run in a 1.5 ~ 2.0% agarose gel containing formaldehyde according to Sambrook *et al.*(1989). A 5X Formaldehyde gel running (FGR) buffer (0.1M MOPS, 40 mM sodium acetate, 5 mM EDTA,pH 8.0) was prepared, adjusted to pH 7.0 with 5 N NaOH, stored at 4°C. Agarose was dissolved in deionized water to a concentration of 2.4% (if a final concentration of 1.5% wanted) or 3.2% (if a final concentration of 2.0% wanted) by heating in a microwave. The solution was stirred for 1 minute with a magnetic stirrer and then cooled to 60°C in waterbath. A 150 ml of RNA gel was quickly made on a warm (50 -60°C) heating plate while stirring by mixing 93.75 ml of the disolved agarose gel (2.5% or 3.2%), 26.76 ml of 37% (w/v) formaldehyde (final concentration: 2.2 M), 29.43 ml of 5X FGR buffer (final concentration: 1X). The gel was cast in the fume hood and left for 1 hour before using. The following were mixed in a 0.5 ml microfuge tube: 4.5 ul RNA (up to 20 ug), 3.5 ul 37% formaldehyde, 2.0 ul 5X FGR buffer, 10 ul deionized formamide and 2.0 ul loading buffer (50% glycerol, 1 mM EDTA, pH 8.0, 0.25% bromophenol blue, 0.25% xylene cyanol FF, DEPC treated). The sample was incubated for 15 minutes in a 65°C waterbath in order to destroy the secondary structure of the RNA, then chilled on ice. 1 ul of ethidium bromide (10 mg/ml) was also added to the RNA mixture to permit visualization under UV. The gel was prerun for 5 minutes at 5 V/cm in 1X FGR buffer, then the RNA mixture was loaded into the wells and the gel was run at 3 - 4 V/cm until the bromophenol blue migrated to 8 cm.

Probes

1.The DNA Probes

We requested and kindly received the following cDNAs from a number of key *Drosophila* labs. *Hunchback* genomic DNA (pE 8), *nanos* (pN5), *Bic-D* (c685) from Dr.Gary Struhl, Howard Hughes Medical Institute. *Nanos* cDNA (pN5)(a 1.8 Kb SalI - XhoI fragment in the pNB40 vector) and *oskar* cDNA from Dr. Ruth Lehmann, Whitehead Institute for Biomedical Research, MA. *Oskar* cDNA (p1952,a 1.9 Kb EcoRI fragment in pGEM1), *bicoid* cDNA (p1128, a ~ 2.4 Kb EcoRI - Hind III fragment in pGEM2) and *exu* cDNA from Dr. Paul Macdonald, Stanford University. *Vasa* cDNA (v1.3, a 1.3 kb EcoRI fragment in plasmid bluescript SK +/-) from Dr. Paul Lasko, McGill University. *Bic-D* cDNA (pc15a^{+}, a 2.9 Kb EcoRI fragment in plasmid bluescript BS^{+}) from Dr.Beat Suter, McGill University. *bicoid* cDNA (c53.46.6) and *hunchback* genomic DNA (pE8) from Dr.C.Nusslein-Volhard, Max Planck Institute for Entwicklungsbiologie, Germany. The antisense PRD-repeat, 5'CGG(A/G)TGCGG(A/G)TGCGG(A/G)TG 3', was artificially synthesized by the DNA Laboratory, Faculty of Medicine, University of Manitoba. Mouse β-actin cDNA was obtained from Dr. Ross McGowan, Dept.of Zoology, University of Manitoba; λ HindIII digested fragments (Phamarcia) was also used as probe to detect the RNA ladder on the Northern blots.

2. Purification of DNA Fragment from Agarose Gel with DEAE Membrane

The *Drosophila* cDNA inserts were cut out from the vector with appropriate restriction enzymes and purified from agarose gel. To monitor the digestion, a small aliquot of the digest (usually containing 100 - 150 ng of DNA) was run in a DNA checking gel along with the un-cut total vector DNA containing the cDNA insert as a control and also with λ HindIII digest as a marker to check if the DNA was completely cut. If it was not fully cut then more enzyme was added and the checking was repeated until the DNA was completely digested. Then the DNA digest was then run in a 1 - 2% agarose gel and the DNA inserts of interest were individually purified from the agarose gel with a DEAE membrane (Mandel Sci.Co.Ltd) and finally a small aliquot of the purified DNA was run in an agarose gel again to make sure that the purified DNA was the wanted DNA fragment and there was no contamination of other DNA fragments. The purified DNA fragments were used as probes for Southern and Northern blot hybridizations. The detailed procedure for the purification of individual DNA fragments from agarose gel with DEAE membrane is presented below.

The DNA digest was usually run in a wide loading well of 1% agarose gel at 80 volts. If a DNA fragment less than 1 Kb was to be purified or two DNA fragments that are close in size are to be separated, a 2% agarose gel was used. When the bromophenol blue had migrated half way down the gel, the gel was taken out of the gel box and stained in 1X TAE solution containing 0.5 ug/ml ethidium bromide for 15 minutes and destained in 1 X TAE solution for 15

minutes and then the gel was viewed under long wave length of UV (302 nm). The DNA band of interest was located and a line was cut in the gel with a razor blade just ahead (~2-3 mm) and immediately behind the DNA band and then the UV was turned off. Strips of 1 cm high DEAE membrane of the same length as the cut line in the gel were cut and presoaked in dd H_2O. One membrane was put into each cut line with wide-tipped tweezers. The gel was put back to the gel box and more 1X TAE running buffer was poured into the gel box to just cover the gel and also remove any air bubbles in the wells. The DNA was run an additional 10 to 15 minutes and the gel was viewed under long wave length of UV to make sure all the DNA band of interest was bound to the DEAE membrane and no longer be visible in the gel. The DEAE membrane containing DNA was taken out of the gel and rinsed in ddH_2O to remove any agarose pieces. Then the membrane was put into a sterile 1.5 ml microfuge tube and completely covered by adding 500 ul high salt elution buffer (1.0 M NaCl, 50 mM Tris.Cl pH8.0, 10 mM EDTA pH8.0). The tube was incubated at 65°C for 45 minutes to remove the DNA from the membrane. After the incubation, the membrane was removed from the tube with tweezers and placed into another tube and rinsed with 100 ul high salt elution buffer which was then combined with the original high salt elution buffer containing eluted DNA. PCI and CIA extraction were performed once (see the DNA isolation section) and the aqueous phase (about 600 ul) was split into two microfuge tubes. To each tube, 0.2 volumes of 7.5 M ammonium acetate and 2 volumes of 100% ethanol were added, then the tubes were inverted several times

and stored at - 20°C for 30 minutes. The tubes were then centrifuged for 20 minutes at 12,000 g. The supernatant was discarded, and the DNA pellet was washed and dried as usual (see DNA isolation section). Finally, the DNA was dissolved in a total of 50 - 200 ul TE^{-4} (10 mM Tris.Cl,pH8.0, 0.1 mM EDTA).

The concentration of the DNA solution was determined by a dot series test. This was done by mixing 1 ul of 10 mg/ml ethidium bromide water solution with 4 ml 1 % melted agarose gel and spread onto a glass slide. After the gel solidified, 1 ul sample of DNA solutions of known concentrations ranging from 6 ng/ul to 500 ng/ul were dotted on the slide. 1 ul of tested DNA solution was also dotted on the same slide. Once the DNA solution dried, the slide was viewed which UV. The fluorescence intensity of the tested DNA dot was compared with that of the standard DNA dots and the concentration of the tested DNA was determined by selecting the appropriate concentration of the standard that matched the fluorescence intensity.

3. **Transformation of E.coli JM109 with Plasmid and the Amplification.**

To amplify the *bicoid* cDNA p1128, it was introduced into E.coli JM109 (Ampicillin sensitive,Promega P9751). The plasmid containing *bicoid* cDNA was thus amplified and subsequently extracted from JM109.

Competent JM109 cells were prepared using the $CaCl_2$ method (Sambrook, *et al.*,1989) with minor modifications. The procedure was as follows. A single colony (2-3 mm in diameter) was removed from LB-agar (1% bacto-tryptone,

0.5% bacto-yeast extract, 1% NaCl, 1.5% agar,pH 7.0) plate grown for 16 - 20 hours at 37°C and transferred to 10 ml LB culture medium and incubated at 37°C in a vigorously shaking waterbath overnight. Then, 1 ml of the overnight culture was transferred to a 50 ml centrifuge tube containing 25 ml LB culture medium and incubated at 37°C in a shaking waterbath for 1 hour. The concentration of cells was checked by measuring the OD value at 15 minutes intervals until it reached to 0.18 - 0.20, then the 50 ml centrifuge tube were placed on ice for 10 minutes before centrifuging the cells at 4,000 rpm for 10 minutes at 4°C. The supernatant was discarded and the pellet was drained free of fluid by inverting the tube for 1 minute. The pellet was resuspended in 10 ml of ice cold 0.1 M $CaCl_2$ solution and left on ice for 30 minutes. The cells were centrifuged again at 4,000 rpm for 10 minutes at 4°C. The supernatant was discarded, and the tube was inverted and drained for 1 minute. The pellet was resuspended in 1 ml of ice cold 0.1 M $CaCl_2$ solution and left on ice for 30 - 40 minutes. 300 ul of the resuspended cells were transferred into each of two ice cold 1.5 ml microfuge tubes and 1 ng of *bicoid* p1128 plasmid DNA was added to one microfuge tube and 1 ul of TE^{-4} was added to the other tube as a negative control. After gently mixing they were stored on ice for 30 minutes. Then the tubes were incubated in a 42°C waterbath for exactly 90 seconds without being disturbed and then chilled on ice for 2 minutes. Then the 300 ul cell suspension was transferred to a 15 ml centrifuge tube containing 700 ul LB culture medium and incubated at 37°C for 45 minutes with vigorous shaking. This step allowed the transformed bacteria to

recover and express the ampicillin resistance marker encoded by the *bicoid* plasmid p1128. After this incubation, 100 ul of the culture was spread with a sterile bent glass rod on a LB-agar plate containing 25 ug/ml of ampicillin and the rest of 900 ul of culture was spread on another culture plate containing 25 ug/ml of ampicillin. The plates were left at room temperature until the liquid on the agar dried and then inverted and incubated in a 37°C oven overnight. In a successful procedure colonies were found on the experimental plates but not on the negative controls the next morning.

The amplification of *bicoid* plasmid DNA was done using a Magic Megapreps DNA Purification System (Promega, A7300). The JM 109, transformed by *bicoid* plasmid, was grown in 500 ml of LB culture medium containing 25 ug/ml of ampicillin at 37°C overnight with vigorous shaking. The cells were pelleted by centrifugation at 4,000 rpm for 20 minutes at 4°C in two 250 ml centrifuge bottles. The cell pellet was resuspended in 30 ml of the kit's Cell Resuspension Solution and 30 ml of the kit's Cell Lysis Solution was added and mixed thoroughly by inversion. The cell suspension then became clear and viscous. Subsequently 30 ml of the kit's Neutralization Solution was added and mixed immediately by inversion several times. After centrifuging at 4,000 rpm for 15 minutes, the clear supernatant was filtered with a Whatman No.1 filter paper into another new 250 ml centrifuge tube. The DNA was reprecipitated by mixing in 0.6 volume of isopropanol, and centrifuged at 14,000 g for 5 minutes. The DNA pellet was resuspended in 5 ml of TE^{-4} buffer and 20 ml of the Magic Megacolumn

Purification Resin was added to the DNA solution, swirled to mix. The Megacolumn tip was inserted into the vacuum manifold. The resin /DNA mix was transferred into the Megacolumn. A vacuum was applied to pull the resin/DNA mix into the Megacolumn. To ensure that all of the DNA/resin mix was transferred to the Megacolumn, 25 ml of the Column Wash Solution was added to the bottle that has contained the DNA/resin mix, swirled and immediately poured into the Megacolumn. Another 25 ml of Column Wash Solution was added to the Megacolumn and suction was applied to draw the solution through the Megacolumn. The resin was rinsed by adding 10 ml of 80% ethanol to the Megacolumn and a vacuum was applied again to draw the ethanol through the Megacolumn and the suction was continued for an additional 10 minutes in order to dry the resin. The Megacolumn was removed from the vacuum manifold and placed in the Reservoir (50 ml screw cap tube). 3.0 ml of preheated (65°C) TE^{-4} was applied to the Megacolumn and left for 1 minute. To elute the DNA, the Megacolumn/Reservoir was centrifuged at 4,000 rpm for 5 minutes with a Beckman JA-17 rotor. The Megacolumn was removed and the plasmid DNA was stored in the capped reservoir. This protocol yielded 2.5 mg of *bicoid* p1128 DNA.

4.**Probe Labelling.**

A.Random Primer Labelling

The random primer labelling method is widely used for DNA labelling (Sambrook,*et al.*,1989). The template DNA is denatured by boiling and then bound to molar excess of oligonucleotides which initiates the DNA synthesis catalysed by the Klenow fragment of E.coli DNA polymerase I resulting in single DNA strand of 400 - 600 nucleotides being produced. All the *Drosophila* DNA probes I used were radio-labelled by this method following the protocol of the Primer-a-Gene® Labelling System (Promega).

For each labelling, 25 ng of DNA in 5 - 10 TE^{-4} was boiled for 3 minutes in a 1.5 ml microfuge tube and then quickly put back on ice. This was then mixed with 10 ul of 5X labelling buffer (Promega U1151, 250 mM Tris.HCl, pH 8.0, 25 mM $MgCl_2$, 10 mM DTT, 1 M HEPES, pH 6.6, and 26 A_{260} units/ml random hexadeoxyribonucleotides), 2 ul mixture of dGTP,TTP,ATP (500 uM each), 0.4 ul of 50 mg/ml of nuclease free BSA, 5 ul of [α-^{32}P] dCTP (Du Pont 10 uCi/ul), 5 units of Klenow enzyme, sterile dd H_2O was added to bring the total volume to 50 ul. The reaction was carried out at 37°C for 60 minutes. The tube was put on ice and 4 ul of renatured salmon sperm DNA (10 mg/ml), 4 ul of 100 mM spermine.4HCl and 140 ul of H_2O were added to the tube, and left on ice for 15 minutes. Centrifugation at 12,000 g for 10 minutes yielded a pellet which was then washed twice with 200 ul TE^{-4}+1mM spermine.4HCl. Then 250 ul TE^{-4}+0.5 M NaCl was added and the tube was boiled for 6 minutes and then quickly put on

ice. The DNA was now ready for hybridization. Usually, the probe radioactivity was measured as high as 200,000 ~ 600,000 counts/2ul/minute.

B.5'End Labelling of the Synthetic PRD-repeat by Phosphorylation

The PRD-repeat was labelled by 5'end labelling by phosphorylation. The synthetic PRD-repeat does not have a phosphate group at its 5' terminus and is therefore easily labeled by transfer of the γ-^{32}P from [γ-^{32}P]ATP using the enzyme bacteriophage T4 polynucleotide kinase (Sambrook,*et al.*,1989).

In a 0.5 ml microfuge tube, the following was added: 0.5 ul of PRD-repeat (10 pmoles/ul); 2.0 ul of 10 X T4 polynucleotide kinase buffer (10X: 0.5M Tris.Cl,pH 7.6; 0.1 M $MgCl_2$, 50 mM dithiothreitol, 1mM spermidine HCl, 1 mM EDTA,pH 8.0); 5 ul of [γ-^{32}P]ATP (10 mCi/ml,sp.act. 5000 Ci/mmole,Amersham); 1.0 ul of T4 polynucleotide kinase (BRL,10 u/ul); deionized H_2O was added to bring the volume to 20 ul. The reaction was incubated at 37°C for 45 minutes and was stopped by adding 2 ul of 0.5 M EDTA (ph 8.0). The tube was then put on ice until use. In this experiment, the labelled probe was not seperated from the free radioactive nucleotide, instead, the whole mixture was mixed with 8 ml of hybrydization mixture as used for Southern blot hybrydization.

Hybridizations.

(1).Southern Blot Hybridization

Appropriate amounts of *Rhodnius* and *Drosophila* genomic DNA were subjected to EcoR I (Pharmacia) digestion for 1 h at 37°C oven. In order to monitor the digestion, usually 10 ul of digestion mixture containing the genomic DNA (about 0.5 ug), EcoRI (about 2 - 3 units) and 1X reaction buffer was mixed with 0.5 ug of pGEM. After the digestion, these test digestion mixtures were run in 1% agarose gel along with 0.5 ug of pGEM that was not digested by any enzyme and also with 0.5 ug of pure pGEM DNA that is digested by EcoRI. If the pGEM in the test mixture was completely digested as viewed under UV after being stained, the overall digestion was judged complete. The digested genomic DNA was then precipitated with ammonium acetate and washed , dried, redissolved as before. The DNA was immediately electrophoresed in 1% agarose gel with 10 ug per lane at 34 volts overnight. Usually a 25 ul of λ HindIII digest (100 ng/ul) was also run in the same gel.

Once the bromophenol blue had migrated 15 cm, the gel was removed and stained in 0.5 ug/ml ethidium bromide/water solution for 20 minutes with occasional agitation. The gel was then destained in dd H_2O for 20 minutes. The gel alongside a ruler was photographed under a UV transluminator. The gel was then soaked in 1 litre of 1X Southern denaturing buffer (4X stock: 2.4 M NaCl, 0.8 M NaOH) for 40 minutes with occasional agitation to denature the double stranded DNA in the gel. After the denaturation, the gel was transferred to a

Southern Blot Box. This box was built in a storage box (40 X 28 X 15 cm) with spongy matrix at the bottom of the box. 20 X SSC (1X SSC: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.5) was poured into the box to a level 1 cm below the top surface of the spongy matrix. Two layers of Whatman No.1 filter paper were wetted with dd H_2O and placed onto the surface of the matrix, one by one with no air bubbles. The gel was slowly placed onto the filter paper and any trapped air bubbles were squeezed out gently with a wetted Wharton bottle. A nylon MSI membrane (Fisher Scientific) was cut a little larger than the gel, wetted, and placed so as to cover the whole gel and again any air bubbles were squeezed out. Strips of discarded X-ray film were placed along the edges of the gel to act as a barrier. Two sheets of Whatman No.1 filter paper were cut to appropriate size, wetted, and placed over the MSI membrane individually avoiding trapping air bubbles. This was followed by putting a layer of paper towel (4 - 5 inches thick) on the Whatman filter paper and a light weight of 200 grams was put onto the paper towels. The 20 X SSC solution thus was drawn from the bottom of the gel and moved upward through the gel by capillary action through the paper towels, the denatured DNA will move along with the SSC solution and becomes bound to the MSI membrane. The Southern transfer was left overnight.

Then the paper towels and the filter paper were removed carefully. A water proof marker pen was used to mark the position of each well of the gel on the MSI paper, and also the position of the lane containing the DNA sample, and the date. The MSI membrane was removed from the gel and rinsed briefly in dd

H_2O, and blotted dry the membrane with paper towels. The MSI membrane was baked in a 80°C oven for 2 hours. The MSI membrane was then washed in a 500 ml of washing solution containing 0.1 X SSC and 0.1% SDS for 40 minutes. The membrane was then placed into a sealed decosonic bag containing 25 ml of prehybridization mix (6X SSC, 10X Denhardts(1X: 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% ficoll), 500 ug/ml single stranded Salmon sperm DNA, 0.5% SDS) at 42°C oven with gentle shaking overnight. The blot was taken out from the oven and stored in 4°C refrigerator in preparation for hybridization.

The *Drosophila* cDNA probe was radio-labelled by random primer labelling (Promega, Primer-a-Gene® labelling system). The specific activity of the labelled probe was measured by adding 2 ul of labelled DNA solution into a plastic vial containing 1 ml of biofluoro which is then put in a Liquid Scintillation System (Nuclear-Chicago Corp., 720 series) to measure the counts/minute of radioactivity. The prehybridization mixture was removed out of the bag containing the blot replaced with 10 ml of hybridization mixture (1 M NaCl, 0.12 M Tris,pH 8.0, 8 mM EDTA, 1X Denhardts, 100 ug/ml of single stranded salmon sperm DNA, 0.5% SDS) containing the radioactive probe solution at the concentration of 4 X 10^6 cpm. The hybridization was done routinely at 55°C overnight in a shaking waterbath.

Subsequently, the blot was taken out of the bag and washed in 1 L of washing solution. For most *Drosophila* probes, the washing was as following

(exceptions are noted in figure legends): first wash: 5X SSC, 0.1% SDS, room temperature,20 minutes; 2nd wash: 5X SSC, 0.1% SDS, 57~58°C 20 minutes; 3rd wash: 2X SSC, 0.1% SDS, 57~58°C,20 minutes; 4th wash: 2X SSC, 0.1% SDS,57~58°C,20 minutes. After washing, the blot was dried with paper towels and wrapped with saran-wrap. The blot was then put into an X-ray Exposure Holder (Eastman Kodak Co.) in contact with an X-ray film (Eastman Kodak Co. Cat No.165 1454) in darkness. The film was exposed at -75°C overnight for 3 days and was then developed in Kodak GBX developer and replenisher for 4 minutes at room temperature, fixed in Kodak rapid fixer with hardener for 4 minutes, then washed in tap water for 15 minutes.

To determine the size of the bands that are detected on the X-ray film, the migrating distance of each band of λ HindIII digest was determined by checking the photograph of the gel, since the migrating distance of DNA is reversibly proportional to the $\log_{10}$ of the number of the DNA base pairs. A linear relationship was obtained between the $\log_{10}$ value of the size of each fragment of λ HindIII and it migrating distance. The migrating distance of the detected band could be measured on the X-ray film and the corresponding $\log_{10}$ value could be measured from the standard λ HindIII graph, and finally the size of the detected band was calculated.

The blots could be stripped and reused. This was done by washing the blots in Southern denaturing buffer at 42-45°C for 30 minutes and then in 1X Southern neutralizer (4X stock:1.0 M Tris.Cl, 2.4 M NaCl) at 65°C for 45 minutes.

The blot was washed in 1 L of washing solution (0.1X SSC, 0.1% SDS) at 65°C for 30 minutes. Then the prehybridization step was repeated and the blots were stored at 4°C refrigerator until next use.

(2).**Northern Blot Hybridization.**

The procedure for Northern blot hybridization was basically similar to that of Southerns with the exception of a few steps. Usually, an RNA marker (GIBCO) was also run simultaneously with the RNA sample. Along with *Rhodnius* ovariole RNA, *Drosophila* female RNA was run as a positive control and *Rhodnius* testis RNA was run as a negative control. After electrophoresis, the gel was rinsed with large volume of deionized water at least 3 times to remove the formaldehyde. The subsequent procedure of Northern transfer through the washing step is exactly the same as that of Southern blot hybridization.

To calculate the size of the RNA band from the Northern blot, part of membrane that contains the RNA marker lane was cut out and probed individually with radio-labelled λ HindIII digest to detect each band of the RNA marker on X-ray film, since the RNA marker is made by *in vitro* transcription of different fragments of λ phage (GIBCO,5620SA) and the RNA marker was not properly detectable using ethidium bromide. The migrating distance was measured directly on the X-ray film and a similar standard graph was made as I did for λ HindIII digest standard graph for the Southern blot.

To reuse the Northern blot, the blot was washed at 65°C for 1.5 - 2 hours

in a washing solution (5 mM Tris.Cl, 0.20 mM EDTA, 0.05% Di-Sodium pyrophosphate, 0.1 X Denhartds) and rinsed in 2 X SSC ,incubated in prehybridization mixture (same as for Southerns) overnight at 42°C with gentle shaking. The blot was then stored in 4°C refrigerator until needed next.

RESULTS

Isolation of Genomic DNA

Figure 3A shows the check gel of the genomic DNAs isolated from *Rhodnius* ovariole and *Drosophila melanogaster* adults. A tight band in each of the sample lanes showed the isolated DNA is not degraded and in high molecular weight form.

Southern Blot Hybridization

In this experiment, the cDNAs of *Drosophila* gene *Bic-D* (p15a), *nanos* (N5), *oskar* (p1952), *vasa* (v1.3) and *bicoid* (p1128) were used as probes for the Southern blot hybridization.

Nanos:

Figure 4 A shows the Southern blot probed with *nanos* cDNA. The 9.7 kb EcoRI fragment containing *Drosophila nanos* gene was found in the *Drosophila*

genomic DNA lane (Wang and Lehmann,1991) but no signal was found in the *Rhodnius* genomic DNA lane under these experimental condition (see the figure legend).

Bic-D:

Figure 4 B shows the Southern blot probed with *Bic-D* cDNA. The 13.6 kb and 6.1 kb EcoRI fragements containing *Drosophila Bic-D* gene were detected in the *Drosophila* genomic DNA lane (Wharton and Struhl,1989) but no signal was found in the *Rhodnius* DNA lane under these experimental condition (see the figure legend).

Oskar:

Since the *oskar* cDNA probe always resulted in a high background, it was not possible to determine whether or not there was a hybridization signal in the *Rhodnius* genomic DNA distinguishable from the background (data not shown).

Vasa:

Figure 5 A shows the Southern blot probed with *vasa* cDNA (V1.3). Eight hybridization bands were detected in the *Rhodnius* genomic DNA lane while the signal from the *Drosophila* DNA lane was too strong to distinguish the individual bands, presumedly due to the relatively low hybridization and washing stringency compared to the *Drosophila* DNA sequence (see the figure legend).

Bicoid:

When the Southern blot was probed with *bicoid* full length cDNA p1128, two hybridization bands of 4.6 kb and 3.2 kb were detected in *Rhodnius* genomic DNA under the washing condition of relatively low stringency (Fig.6 A). Increasing the washing stringency resulted in a loss of the 4.6 kb band but the strong hybridization signal from the 3.2 kb band remained. The 8.7 kb EcoRI fragment of *Drosophila* genomic DNA containing the *bicoid* gene (Berlith, *et al.*,1988) was also revealed in the *Drosophila* DNA lane.

Isolation of RNA

Figure 3 B shows the check gel of the isolated RNA from *Rhodnius* ovariole, testis and *Drosophila melanogaster* females. The 18 S and 28 S ribosomal RNAs were well stained as visible in the *Rhodnius* ovariole RNA lane however the 28 S rRNAs of *Drosophila* RNA and *Rhodnius* testis RNA were not visible.

Northern Blot Hybridization

Since positive signals were found on *Rhodnius* genomic Southern blots when probed with *vasa* and *bicoid* cDNA, Northern blot hybridization was performed by using *vasa* cDNA V1.3 and *bicoid* cDNA p1128 as probes in order to determine whether the sequences homomlogous to *vasa* and *bicoid* detected in *Rhodnius* genomic DNA were also expressed specifically in *Rhodnius* ovariole system.

As shown in figure 5 B, when probed with *vasa* V1.3, two faint bands sized 1.8 Kb and 0.3 Kb were detected in the *Rhodnius* ovariole RNA lane but not in the *Rhodnius* testis lane while the 2.0 kb *vasa* mRNA was found in *Drosophila* female RNA lane (Lasko and Ashburner, 1988) under this washing condition (see the figure legend).

A strong hybridization signal sized 0.53 Kb and a faint band sized 2.8 Kb was found only in the *Rhodnius* ovariole RNA lane and not in the testis RNA lane when the RNA blot was probed with *bicoid* p1128 cDNA (Figure 6 B). The 2.6 kb *bicoid* mRNA (Berleth, *et al.*,1988) was also detected in the *Drosophila* female RNA lane. These results from Northern blot hybridization indicated that *bicoid* and *vasa* homologous sequences are not only present in *Rhodnius* genomic system but also expressed in *Rhodnius* ovariole system.

Characterization of *bicoid* cDNA Hybridization

The *bicoid* full length cDNA p1128 that I used as a hybridization probe contains several functional subregions including the *bicoid* class homeobox (Berleth *et al.*,1988), the PRD-repeat (Frigerio *et al.*,1986), the M-repeat (McGinnis *et al.*,1984) in addition to the major coding region. In order to identify the subregions within the *bicoid* cDNA that are reponsible for the hybridization signals with the Southern and Northern Blots, p1128 cDNA was cut with several restriction enzymes into several fragments containing those subregions and these fragments were separated in and purified from agarose gel and used to reprobe

the Southern blots and Northern blots with the same washing stringency as before. These purified fragments were (figure 7 A): A. a 252 bp EcoRI-HinfI fragment (position: 0 - 252 nt) containing the PRD-repeat (position: 32 - 122 nt); B. a 211 bp HinfI-AvaII fragment (position: 252 - 463 nt) containing the *bicoid* class homeo box (position: 272 - 452 nt); C. a 262 bp AvaII fragment (position: 463 - 725 nt); D. an about 1.7 kb SalI-HindIII fragment (position: 738 - ~2,400 nt) containing the M-repeat and rest of the *bicoid* coding region.

As shown in Figure 7 B and C, no signal was detectable on the Southern and Northern blots when probed with the DNA fragments containing *bicoid* class homeobox, M-repeat and majority of the rest of the coding region. However, when the fragment A containing the PRD-repeat was used as probe, the 4.6 kb and 3.2 kb bands in *Rhodnius* genomic DNA lane were still detected on the Southern blot and the 0.53 kb band in *Rhodnius* ovariole RNA lane was also detected on the Northern blot.

Probing the same Northern blots with the mouse β actin cDNA revealed a tight band sized ~2.0 kb in the *Rhodnius* ovariole RNA lane indicating the RNA was not degraded (figure 7C), however β-actin cDNA failed to detect any band from *Rhodnius* testis RNA (Data not shown).

Analysis of the PRD-repeat Hybridization in *Rhodnius* System.

Since the *bicoid* cDNA fragment A containing the PRD-repeat hybridized strongly to *Rhodnius* genomic DNA and ovariole RNA, a synthetic oligonucleotide consisting of 18 nucleotides including three antisense PRD-repeats: 5'CGG(A/G)TG 3' was used as probe to reprobe the *Rhodnius* genomic Southern blot and ovariole RNA blot in order to further explore the existence of PRD-repeat in *Rhodnius* system. The PRD-repeat oligomer still hybridized to the 4.6 kb and 3.2 kb *Rhodnius* genomic DNA EcoRI fragments (figure 7B). When the washing stringency is increased the signal from the 3.2 kb band failed to be detected but the the signal of the 4.6 kb band was still present (figure 7B). Surprisingly, the PRD-repeat oligomer did not hybridize to the *Rhodnius* ovariole RNA (Figure 7 C).

Figure 3.

A. The DNA check gel of the isolated genomic DNAs from *Rhodnius* ovarioles (Lane 1) and *Drosophila* adults (Lane 2). ~ 0.5 ug of *Rhodnius* genomic DNA and ~0.3 ug of *Drosophila* genomic DNA along with ~ 1.5 ug of λ DNA-HindIII digest (Lane M) were electrophoresed in a 1% agarose gel in TAE buffer, and stained in 0.5 ug/ml ethidium bromide water solution.

B. The RNA check gel of the isolated total RNAs from *Drosophila melanogaster* females (Lane 1), *Rhodnius* testes (Lane 2) and *Rhodnius* ovarioles (Lane 3). 5 ug of total RNAs from *Drosophila* females and *Rhodnius* testes and 2 ug of total RNA from *Rhodnius* ovarioles along with 3 ug of the RNA ladder (GIBCO, Cat No.5620SA) were electrophoresed in a 1.5% formaldehyde RNA denaturing gel. Each RNA sample was mixed with 1 ul of ethidium bromide (10 mg/ml in water) prior to electrophoresis.

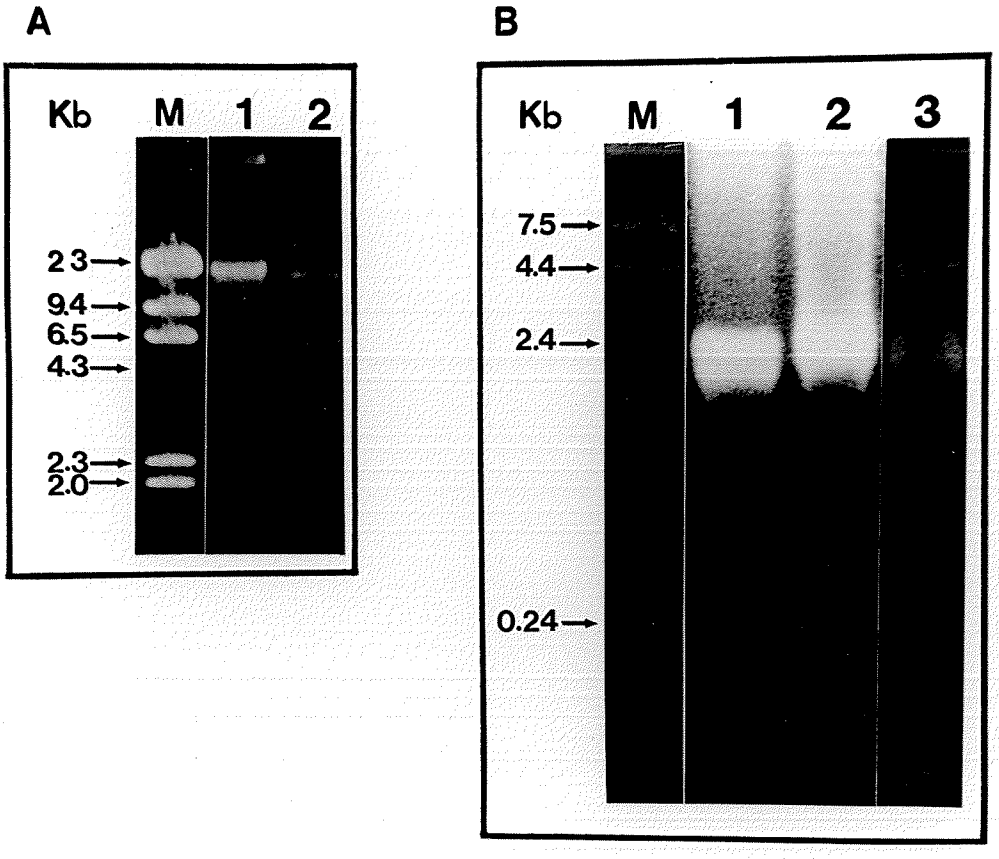

A
Kb
M
1
2
23
9.4
6.5
4.3
2.3
2.0
B
Kb
M
1
2
3
7.5
4.4
2.4
0.24

Figure 4

A. Southern blot hybridization using *nanos* cDNA pN5 as a probe. 10 ug of DNA from *Drosophila* adults (Lane 1) and *Rhodnius* ovarioles (Lane 2) were electrophoresed in a 1% agarose gel, transferred to MSI nylon membrane and hybridized to *Drosophila nanos* cDNA pN5 probe. The hybridization was carried out at 60°C in a routine hybridization mixture (see the methods) overnight. The washing was done routinely as indicated in the methods but at 62°C. Autoradiographic exposure time was 16 hours.

B. Southern blot hybridization using *Bic-D* cDNA p15a as probe. 10 ug of DNA from *Drosophila* adults (Lane 1) and *Rhodnius* ovarioles (Lane 2) were electrophoresed in a 1% agarose gel, transferred to MSI nylon membrane and hybridized to *Drosophila Bic-D* cDNA p15a probe. The hybridization was carried out at 50°C overnight in a routine hybridization mixture (see the methods). The washing was done at 50°C, twice in 2X SSC, 0.1% SDS, for 20 minutes each; once in 0.5X SSC, 0.1% SDS, for 20 minutes; once in 0.2X SSC, 0.1%SDS, for 20 minutes. The autoradiographic exposure time was 5 days.

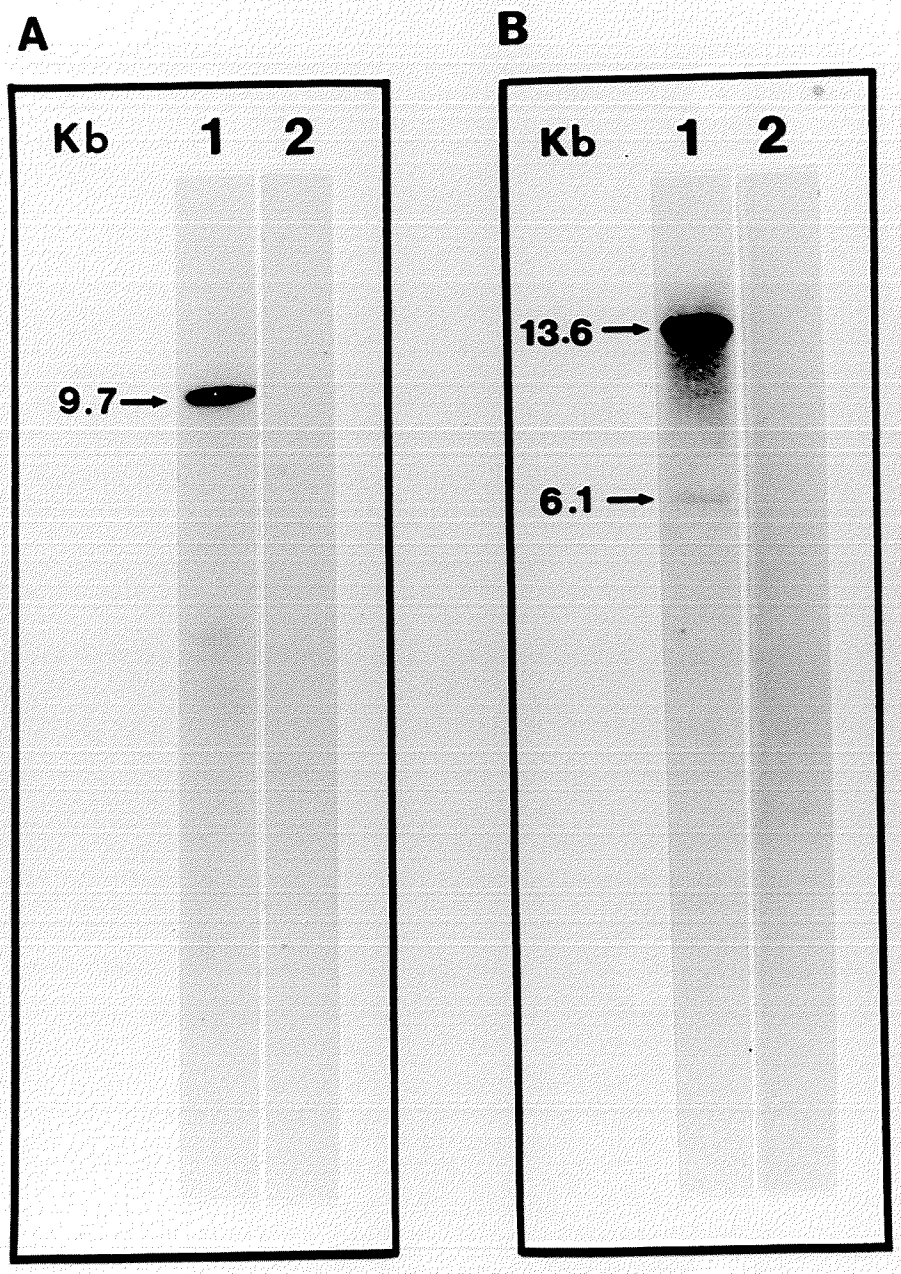

A
Kb
1
2
9.7
B
Kb
1
2
13.6
6.1

Figure 5

A. Southern blot hybridization using *vasa* cDNA V1.3 as probe. 10 ug of DNAs from *Drosophila* adults (Lane 1) and *Rhodnius* ovarioles (Lane 2) were electrophoresed in a 1% agarose gel, transferred to MSI nylon membrane and hybrydized to the *Drosophila vasa* cDNA V1.3 probe. The hybridization was carried out as indicated in the methods. The washing was done at 55°C twice in 2X SSC,0.1% SDS, for 20 minutes each; once in 1X SSC,0.1% SDS, for 30 minutes; once in 0.25X SSC,0.1% SDS, for 20 minutes. The autoradiographic exposure time was 7 days.

B. Northern blot hybridization using *vasa* cDNA V1.3 as probe. 10 ug of total RNA from *Drosophila* females (Lane 1), *Rhodnius* testes (Lane 2) and *Rhodnius* ovarioles (Lane 3) were electrophoresed in 1.5% agarose formaldehyde RNA denaturing gel, transferred to MSI nylon membrane, and hybridized to *Drosophila vasa* cDNA V1.3 probe. The hybridization was carried out at 59°C overnight in a routine hybridization mixture. The washing was done once in 2X SSC,0.1% SDS, at room temperature, for 20 minutes; once in 2X SSC,0.1% SDS, at 59°C, for 20 minutes; once in 2X SSC,0.1% SDS at 62°C, for 20 minutes. The autoradiographic exposure time was 4 days.

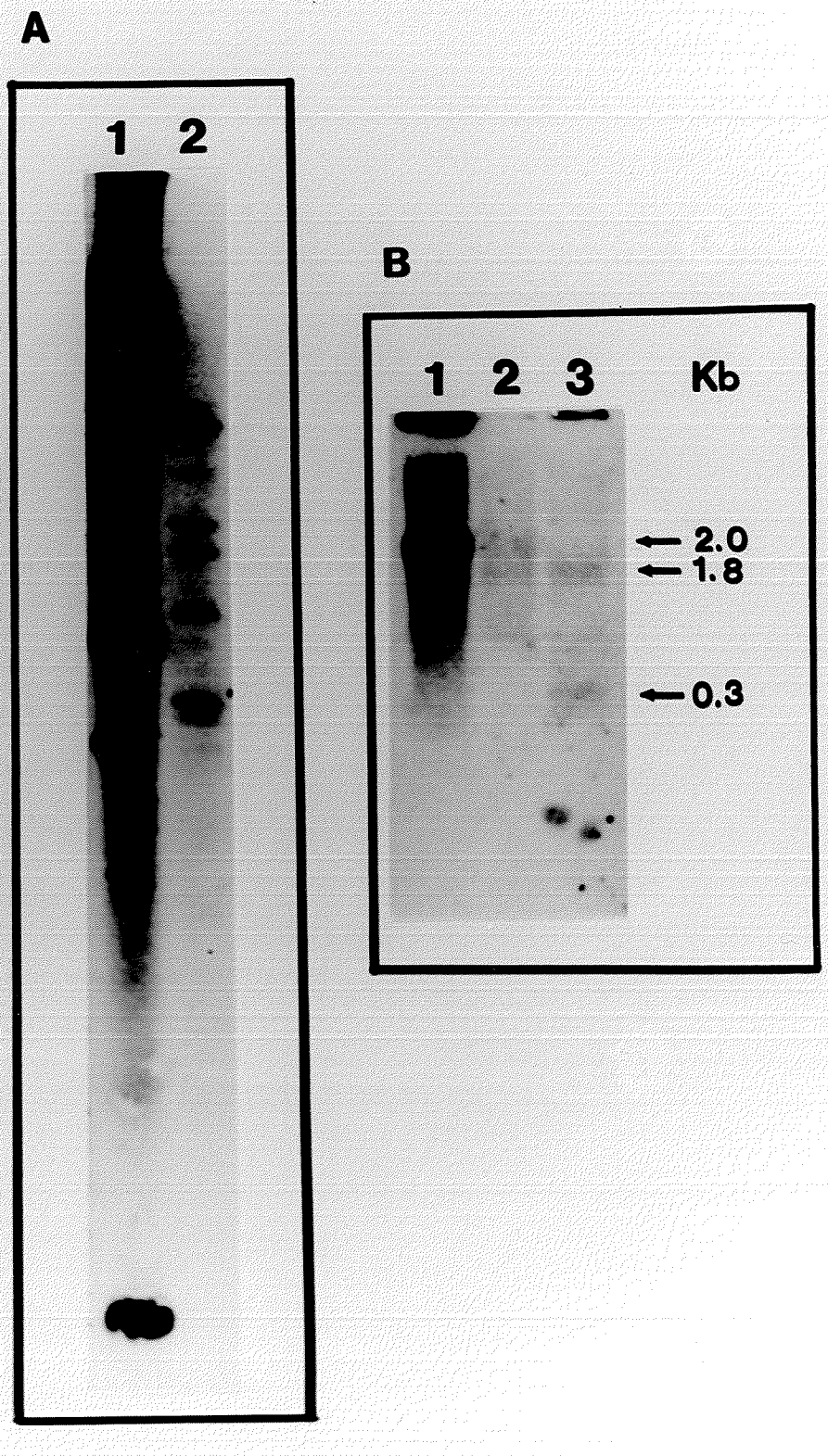

A
1 2
B
1 2 3
Kb
2.0
1.8
0.3

Figure 6

A. Southern blot hybridization using *bicoid* cDNA p1128 as probe. 10 ug of DNAs from *Drosophila* adults (Lane 3) and *Rhodnius* ovarioles (Lane 1,2) were electrophoresed in 1% agarose gel, transferred to MSI nylon membrane, hybridized to *Drosophila bicoid* cDNA p1128 probe. The hybridization was done routinely as indicated in the methods. The washing for Lane 1 was done routinely as indicated in the methods. The washing stringency for Lane 2 and 3 was raised. This was done twice in 2X SSC,0.1% SDS,at 58°c, for 20 minutes; once in 1X SSC,0.1% SDS, at 58°C, for 20 minutes; once in 0.5X SSC,0.1% SDS, at 58°C , for 20 minutes; once in 0.25X SSC,0.1% SDS, at 58°C , for 15 minutes. The autoradiographic exposure time was 16 hours for Lane 1 and 24 hours for Lane 2 and 3.

B. Northern blot hybridization using *bicoid* cDNA p1128 as probe. 10 ug of total RNA from *Drosophila* females (Lane 1), *Rhodnius* testes (Lane 2) and *Rhodnius* ovarioles (Lane 3) were electrophoresed in 1.5% agarose formaldehyde RNA denaturing gel, transferred to MSI nylon membrane, hybridized to *Drosophila bicoid* cDNA p1128 probe. The hybridization was carried out at 59°C in a routine hybridization mixture overnight. The washing was done once in 2X SSC,0.1% SDS, at room temperature, for 20 minutes; once in 2X SSC,0.1% SDS, at 59°C, for 20 minutes; once in 2X SSC,0.1% SDS,at 62°C,for 20 minutes; once in 1X SSC,0.1% SDS, at 62°C, for 20 minutes. The autoradiographic exposure time was 4 days.

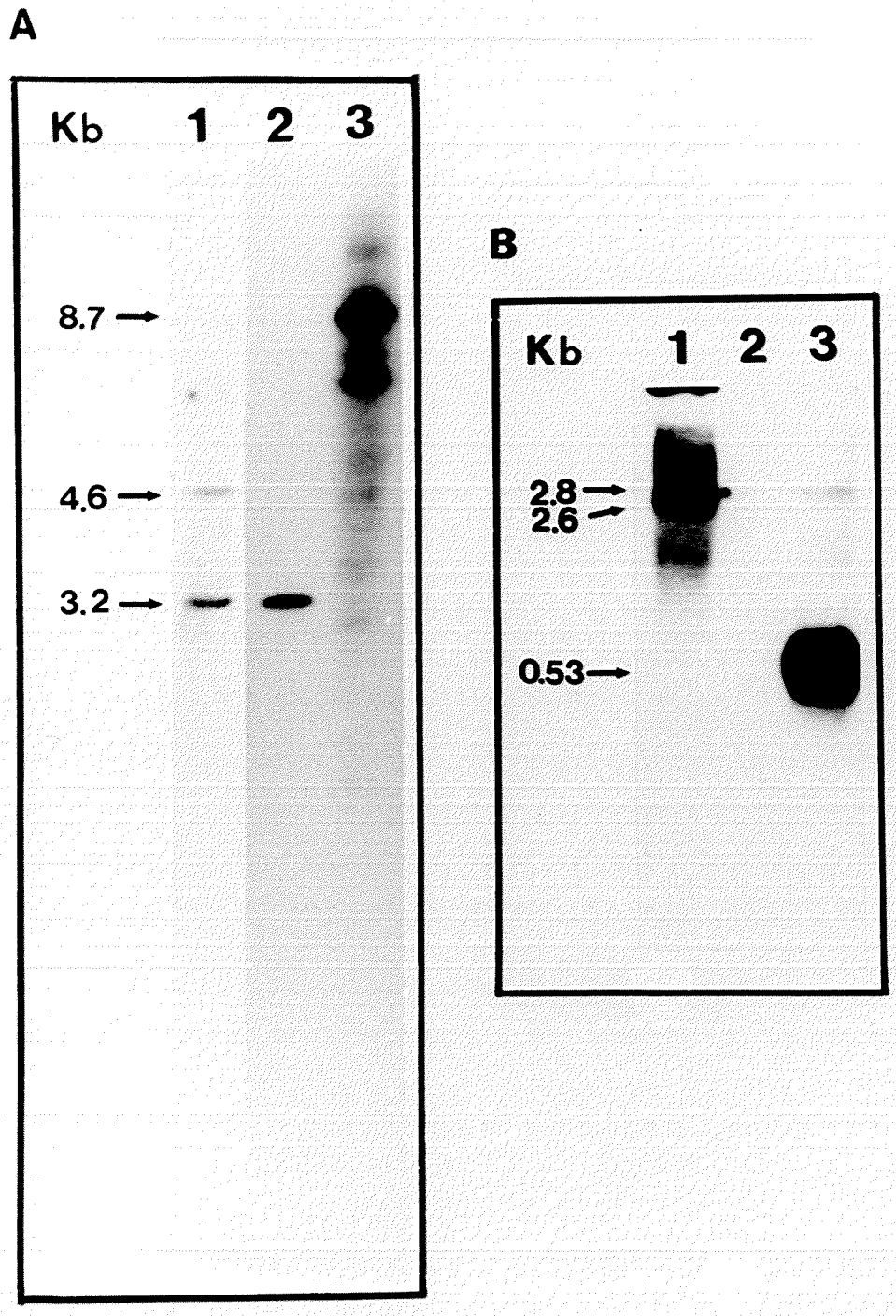
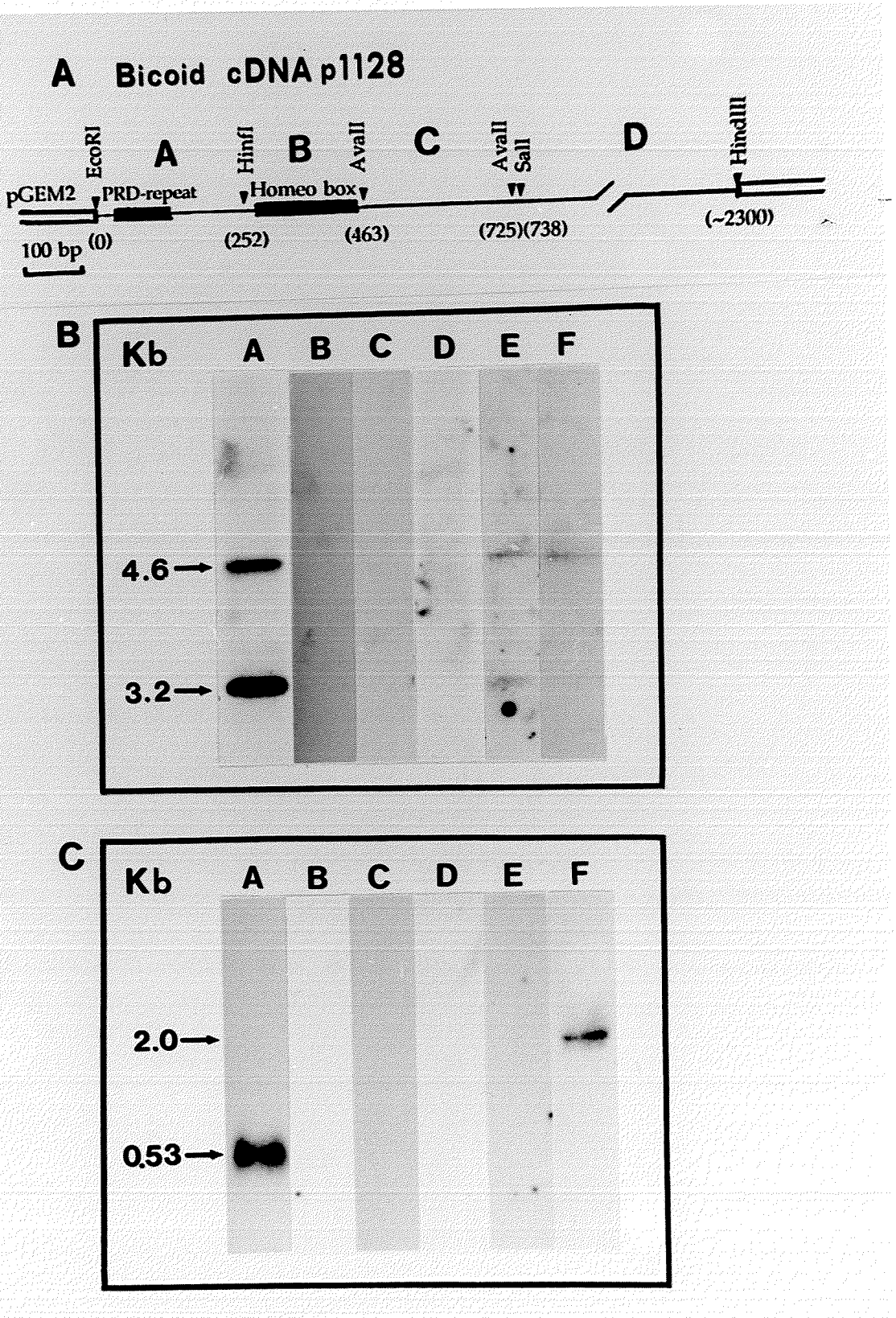

A
Kb
1
2
3
8.7
4.6
3.2
B
Kb
1
2
3
2.8
2.6
0.53

Figure 7

A. A schematic of the *bicoid* cDNA p1128. Fragment A is 252 bp in length containing a 90 bp long PRD-repeat. Fragment B is 211 bp in length containing a 180 bp long homeo box. Fragment C is 262 bp in length. Fragment D is ~ 1.7 Kb in length containing M-repeat and rest of the *bicoid* coding region.

B. Charaterization of *bicoid* hybridization on Southern blots. The same Southern blots as shown in figure 6 containing 10 ug of *Rhodnius* genomic DNA in lane A,B,C and D was hybridized to fragment A, B, C and D resepectively. The hybridization and washing was done routinely as indicated in the methods. The same Southern blot containing *Rhodnius* genomic DNA was also hybridized to a synthetic PRD-repeat probe (Lane E and F) at 53°C in routine hybridization mixture overnight. The washing for Lane E was done once in 5X SSC,0.1% SDS, at room temperature,for 20 minutes; twice in 2X SSC,0.1% SDS, at 55°C,for 20 minutes each. The washing for Lane F was done additionally once in 1X SSC,0.1% SDS, at 55°C, for 20 minutes; once in 0.5X SSC,0.1% SDS,at 55°C,for 20 minutes. The autoradiographic exposure time was 24 hours.

C. Characterization of *bicoid* hybridization on Northern blot. The *Rhodnius* ovariole RNA blot containing 20 ug of total RNA (lane A,B,C,D,and E) was hybridized to fragment A,B,C,D and synthetic PRD-repeat probe respectively and washed at the same condition indicated in figure 7 B. The same blot was also hybridized to mouse β-actin cDNA to detect the integrity of the *Rhodnius* ovariole RNA (Lane F). The autoradiographic exposure time was 24 hours.


A Bicoid cDNA p1128
EcoRI
A
HinfI
B
AvaII
C
AvaII
SalI
D
HindIII
pGEM2
PRD-repeat
Homeo box
100 bp
(0)
(252)
(463)
(725)(738)
(~2300)
B
Kb
A B C D E F
4.6
3.2
C
Kb
A B C D E F
2.0
0.53

DISCUSSION

The Reliability of the Techniques

I have used cross-species Southern and Northern blot hybridization techniques to study the homology between two insect species: *Drosophila melanogaster* (Diptera) and *Rhodnius prolixus* (Hemiptera) at the molecular level. This is the first attempt to apply this approach to *Rhodnius*. The reliability of the techniques used is an important consideration in the interpretation of the results.

The technique for isolating genomic DNA from *Rhodnius* ovarioles was worked out in this research. The proteinase K was found to be very effective to digest the ovariole proteins which were then efficiently separated from the nucleic acids by using at least three phenol extractions. This yielded a ratio of $OD_{260nm/280nm}$ ranging from 1.8 ~ 2.0, the indicative of the purity of DNA, which was further demonstrated by the results of the DNA check gels. The RNAase digestion step was also indispensable for the successful isolation of DNA from the *Rhodnius* ovarioles. Deletion of this step resulted in the presence of at least 8 ~ 9 times as much RNA in sample as there was DNA. This made it impossible to calculate the actual concentration of the DNA. This also indicated that the *Rhodnius* ovarioles were extremely active in RNA synthesis.

Guanidinium thiocyanate - phenol-chloroform extraction was used to isolate RNA. The chaotropic agent guanidinium thiocyanate, which is extremely effective in denaturing proteins and inactivating RNAase, was first introduced by

Chirgwin *et al.* (1979) to isolate biologically active RNA. This method was further modified by Chomczynski and Sacchi (1987) in which phenol-chloroform extraction was used to remove proteins from RNA, replacing the tedious and expensive ultracentrifuge step, which made it possible to recover total RNA from small quantities of tissues and also allowed the simultaneous processing of a large number of samples. Therefore, this method was chosen to isolate RNA from *Rhodnius* ovarioles and testis and *Drosophila* females. Pure and intact total RNA was recovered from *Rhodnius* ovarioles at high yield as shown by the results of the RNA check gel and integrity checking with the mouse β-actin cDNA. However, the *Drosophila* female RNA isolated with this protocol often contains protein as indicated by the ratio of $OD_{260nm/280nm}$. Since mouse β-actin cDNA failed to detect any signal from *Rhodnius* testis RNA, the negative results in the *Rhodnius* testis RNA lane on the Northern blots when probed with *bicoid* cDNA p1128 and *vasa* cDNA v1.3 are questionable.

Since the Southern and Northern blot hybridizations were the major techniques used in this study, a thorough understanding of the parameters influencing nucleic acid hybridization is essential for interpreting the results especially for the cross-species hybridization dealing with different degree of homology between DNA sequences. The hybridization occuring between probes and its target may be estimated with the melting temperature T_m which defined as the temperature when half of the duplex molecules have dissociated into their constituent single strands. T_m is affected by the monovalent cation concentration

(M), the base composition expressed as mole fraction of G and C residues, the length in the nucleotides of the shortest chain in the duplex (L), and the concentration of helix-destablizing agents such as formamide. The following equations of T_m are for the DNA-DNA and DNA-RNA duplexes, valid from pH 5 to 9 and for probes longer than 50 nucleotides (Wahl, *et al.*,1987):

$$T_{mDNA\text{-}DNA} = 81.5° + 16.6 \log M + 41 \text{ (mole fraction G + C)} - 500/L - 0.62(\%\text{formamide})$$

$$T_{mDNA\text{-}RNA} = 79.8° + 18.5 \log M + 58.4 \text{ (mole fraction G + C)} - 820/L - 0.5(\%\text{formamide})$$

From the above equation, the hybridization between probes and its target could be enhanced by raising the monovalent cation concentration, lowering the hybridization temperature, eliminating formamide, and vice versa. My hybridizations were routinely carried out at 55°C and in solutions of 1 M Na^+ without formamide, which maximized the rate of annealing of the probe with its target. The washing was routinely done at raised temperature (ranging from 57°C to 60°C) and decreasing the salt concentration from 5X SSC (~0.8 M Na^+) to 2X SSC (~0.3 M Na^+). This was considered as medium washing stringency. At this stringency, the hybridization signal was interpreted to be due to specific binding of the probes to its homologous sequences in *Rhodnius*. Also, each experiment was repeated routinely two to three times with different batches of nucleic acids and different blots in order to eliminate the possibility of artifact and confirm the results.

Conservation of the Homologous Sequence of *Drosophila* Maternal Genes in *Rhodnius* System

As mentioned in the introduction, the *nanos* gene is specially involved in the determination of abdominal formation in the *Drosophila* system (Lehmann and Nusslein-Volhard, 1991), part of *nanos* gene is shared by another recently cloned *Xenopus* maternal gene Xcat-2 (Mosquera *et al.*,1993), which showed that *nanos* gene was conserved to a certain degree in an evolutionarily higher system like *Xenopus*. Under the experimental condition in this research, *nanos* signal was not detectable in *Rhodnius* genome. Whether or not it is present or the amount present in my nucleic acid preparations is below the detection sensitivity of my methods is unknown.

Bic-D is a maternal gene required for the differentiation of cystocytes into oocyte in *Drosophila* (Steward and Nusslein-Volhard, 1986; Suter and Steward,1991). It encodes a coiled polypeptide with sequence similarity to the tail domain of the myosin heavy chain, the microtubule motor kinesin and intermediate filament proteins (Suter *et al.*,1989; Wharton and Struhl,1989). The *Bic-D* cDNA was chosen as a probe with the expectation that in *Rhodnius* ovarioles there may be genes similar to *Bic-D* to be involved in determination of the differentiation of *Rhodnius* oocyte. As with *nanos*, under the experimental conditions in this research, a *Bic-D* signal was not detectable in *Rhodnius* genome.

The *Vasa* gene encodes a 660 amino acid protein which has 29.1% amino acid identity with murine eukaryotic initiation factor-4A (eIF-4A) including the

eIF-4A ATP-binding site (Lasko and Ashburner,1988). eIF-4A, together with eIF-4B and eIF-4F, functions to recognize the mRNA cap structure and catalyse RNA unwinding (see reference in Lasko and Ashburner,1988). The *vasa* protein is thought to be an RNA binding protein that regulates the translation of localized transcripts required for determination of posterior structures of *Drosophila* embryo (Hay,*et al.*,1990). Eight *vasa* hybridization bands were found on *Rhodnius* genomic Southern blot and two weak bands were found on the *Rhodnius* RNA blot, one at 1.8 kb, the other at 0.3 kb. While a small band like 0.3 Kb from *Rhodnius* ovariole RNA blot seems unusuall, the same blot was also probed with a mouse β-actin cDNA and one tight hybridization band was obtained, indicating the RNA was intact. The 0.3 Kb signal may be a specific splicing or endogenously degraded product from the 1.8 kb RNA. Although the region within the *vasa* cDNA V1.3 that is responsible for the hybridization was not studied in this research, the result shows there are at least a certain amount of homologous sequence conserved in *Rhodnius* and weakly expressed in the *Rhodnius* ovariole.

An important finding of my study is that *bicoid* cDNA p1128 hybridizes strongly to two EcoRI fragments (sized 4.6 kb and 3.2 kb) of *Rhodnius* genomic DNA and the homology of the 3.2 kb fragment to the p1128 is higher than that of the 4.6 kb since the hybridization signal of the 4.6 fragment disappears when the washing stringency is raised while the 3.2 kb signal remains.

Within the *bicoid* cDNA there are also several identified DNA motifs which are: 1. PRD-repeat, a DNA sequence (60 -90 bp) which encodes repetitive histidine

and proline (Frigerio, *et al.*,1986); 2.*bicoid* class homeo box, a DNA sequence that encodes a functional domain that binds to a specific DNA sequences and act as a transcriptional factor (Treisman *et al.*,1992); 3.M-repeat, a DNA sequence encoding repetitive glutamines (McGinnis *et al.*,1984). Further study showed that only the 252 bp fragment A containing the PRD-repeat within the p1128 cDNA was responsible for the hybridization to the two EcoR I fragments in the *Rhodnius* genome. None of the other *bicoid* coding regions, including *bicoid* class homeo box and M-repeat, hybridized to the *Rhodnius* DNA. Synthetic PRD-repeat probe hybridizes to both of the two bands detected before. This strongly indicates that the *Drosophila bicoid* PRD-repeat region is conserved in the *Rhodnius* genome but most of the coding region of *bicoid* gene including *bicoid* class homeo box and M-repeat does not appear to be conserved in *Rhodnius* system or the level of homology is very low.

Since two PRD-repeat hybridization bands were detected from the *Rhodnius* genomic Southern blot, two explanations may be possible: First, the two fragments that hybridize to the synthetic PRD-repeat probe may be from two independent genes in the *Rhodnius* genome, the 4.6 kb fragment contains a real PRD-repeat while th 3.2 kb fragment contains a PRD-repeat homologous sequence but with less homology to PRD-repeat than the one within the 4.6 kb fragment, so the 3.2 kb signal was washed out when the washing stringency was raised ; second, there is only one gene that has PRD-repeat in *Rhodnius* genome but that there is one EcoRI cutting site within the PRD-repeat resulting in the two

hybridization bands detected by the PRD-repeat synthetic probe and also the sequence within the 3.2 kb fragment is less homologous to the PRD-repeat than the sequence within the 4.6 kb fragment.

Interestingly, *bicoid* cDNA p1128 strongly hybridizes to the 0.53 kb RNA fragment and weakly to a 2.8 kb RNA fragment from the *Rhodnius* ovariole RNA blot. My further characterization showed that only the 252 bp fragment A of *bicoid* cDNA containing PRD-repeat specifically hybridizes to the 0.53 kb *Rhodnius* RNA fragment. This strongly indicates that the homologous sequence to the 252 bp fragment A region of *bicoid* cDNA is not only conserved in the *Rhodnius* genome but also expressed in *Rhodnius* ovariole system. Again, the RNA was intact as checked by mouse β-actin cDNA, the 0.53 kb fragment may also be a specific splicing product from the 2.8 kb fragment or endogenously degraded product. The positive results obtained here is from the total RNA from *Rhodnius* ovarioles which also contain an abundance of ribosomal RNA, the 0.53 kb *bicoid* homologous signal is not likely from the rRNA since the sizes of eukaryotic 26-28S, 16-18S, 5.8S and 5S rRNA are 3.8-5.1 kb, 1.5-1.9 kb, ~160 bp and 120 bp, respectively. Even the transfer RNA is 70 -80 bp in length (Darnell *et al.*,1990). The 0.53 kb *bicoid* homologous signal was only detected when probed with the fragment A of the *bicoid* cDNA, so it is not either likely that the positive signal is from non-specific RNA-DNA binding. Therefore, the positive signals from *Rhodnius* RNA blots were interpreted to indicate the ovariole mRNA.

The synthetic PRD-repeat probe failed to hybridize to any RNA fragment

on the *Rhodnius* ovariole RNA blot, especially to the 0.53 kb RNA fragment that is detected by the fragment A containing the PRD-repeat. Combining the result from Southern blot that the 3.2 Kb DNA fragment has higher homology than the 4.6 kb DNA fragment when probed with the whole p1128 cDNA, I interpreted the results to suggest that besides the conserved PRD-repeat in *Rhodnius* genome, there must be some sequence along with the PRD-repeat from the 252 bp *bicoid* cDNA fragment A conserved in the *Rhodnius* genome, probably within the 3.2 kb *Rhodnius* EcoRI DNA fragment, and expressed in the *Rhodnius* ovariole system, and there may be only PRD-repeat conserved in the 4.6 kb EcoRI fragment.

The Possible Role of PRD-Repeat and Other Homologous Sequence of *bicoid* in *Rhodnius* System

Twelve genes in *Drosophila* have been found to contain the PRD-repeat. These are often associated with homeo box, and/or the M-repeat (Frigerio, *et al.*,1986). Among these genes, only two genes have had their biological roles identified so far. One is *bicoid* , which is an indispensable and key gene in the determination of anterior structures (St Johnson and Nusslein-Volhard,1992). The other is the pair-rule gene *paired*, which also contains pair-box and homeo box (Frigerio, *et al.*, 1986, Bopp, *et al.*, 1986). Deletion of this gene will cause the deletion of the embryo segments at a two-segment periodicity (Kilcherr,*et al.*,1986). However, the exact function of PRD-repeat in these genes is still unknown. No PRD-repeat has been found in any other organisms besides

Drosophila until now.

This leads to the question, what is the significance of the finding that the PRD-repeat is conserved in *Rhodnius* ? It has become increasingly clear in recent years that proteins and their genes are further divided into functional domains. In his exon shuffling theory, Gilbert proposed (1978,1986) that these functional domains may be individually recombined and independently assorted to build a new set of functional genes during evolution. The most striking example that illustrates this theory is the gene that codes for the low-density lipoprotein receptor (Sudhof *et al.*,1985). This gene has 18 exons which are actually a combination of several functional groups of exons. Several exons have sequence similarity to regions of epidermal growth factor or the blood clotting factors, some other exons are homologous to a blood protein called complement factor 9. Still other exons encode a signal sequence for the targeting of the receptor to the endoplasmic reticulum membrane, a transmembrane domain for anchoring the receptor in the membrane and a domain to which polysacchride side chains are attached. It seems most likely that this mosaic of domains was assembled by shuffling of exons from different transcription units.

This theory implies the existence of a network of gene sets in each organism in which the genes only share one or several DNA regions that encode special functional protein domain (Frigerio,*et al.*,1986). For example,in *Drosophila* the homeo box is found to be shared by most *Drosophila* developmental genes including *bicoid* and *paired* gene (Treisman *et al.*,1992;Scott,*et al.*,1989). M-repeat (or

opa-repeat) was found within the genes of antennapedia and bithorax complexes (McGinnis *et al.*,1984) and also conserved in the *bicoid* gene (Berlith *et al.*,1988). The PRD-repeat is conserved within 12 *Drosophila* genes including *bicoid* and the pair-rule gene *paired* (Berleth *et al.*,1988; Frigerio *et al.*,1986). The pair box, a DNA sequence encoding a DNA-bind domain (Treisman *et al.*,1991), was also found to be shared by pair-rule gene *paired, gooseberry, Pox meso,*and *Pox neuro* (Bopp *et al.*,1986,1989). Thus it is not surprising to find that both *bicoid* and *paired* gene contain homeo-box, and PRD-repeat sequences.

Another extension of this theory which could explain the existence of PRD-repeat in *Rhodnius* genome is that genes from different species or even different phyla may also share certain functional domains. The dramatic example is the homeo box, an 180 bp DNA sequence encoding a homeo domain which is a DNA binding domain, has been identified genetically to be shared by many genes from *Drosophila, C.elegans,* yeast, mouse and human (Scott *et al.*,1989). The zinc-finger domains, a DNA binding domain encoded by three *Drosophila* segmentation genes *hunchback,Kruppel* and *snail* were found to be conserved within a variety of arthropods and partly also other animal phyla such as Mollusca, Annelida and Vertebrata (Sommer *et al.*,1992). The 3'untranslated region of *bicoid* mRNA from *Drosophila melanogaster* that specifically directs the correct localization of *bicoid* mRNA is also shared by six distant *Drosophila* species in which all the shared sequences can potentially form a large sterotypic secondary structure (MacDonald,1990).

My research has shown that the PRD-repeat is present in the *Rhodnius* genome and possibly also part of a DNA sequence other than the PRD-repeat from the fragment A of the *bicoid* p1128 is not only conserved in *Rhodnius* genome but also expressed in *Rhodnius* ovariole. The significance of this discovery is two fold: First, according to Gilbert's theory, the existence of a PRD-repeat in the *Rhodnius* system suggests that the PRD-repeat is functionally important and conserved as a functional domain during evolution. There may also be a gene set in *Rhodnius* genome since two different hybridization bands were found on *Rhodnius* genomic Southern blots; Secondly, part of DNA sequence other than PRD-repeat in *bicoid* gene also appears to be conserved in the *Rhodnius* genome and expressed in the *Rhodnius* ovariole. This also implies there may be another unidentified DNA domain that is functionally important and shared by both *Rhodnius* and *Drosophila*, and the 0.53 Kb RNA presumedly represents a very small protein in *Rhodnius* ovariole system.

The role of the PRD-repeat is not understood at molecular level. However, one clue that we have is the amino acid histidine which is encoded by the PRD-repeat. Zinc-finger proteins, the first well-characterized eukaryotic positive acting regulatory protein required for RNA polymerase III transcription of the 5S-rRNA genes (see Darnell,*et al.*,1990), has nine repeated domains that contain cysteine and histidines spaced at regular intervals forming finger-like loops. These domains of the protein are specifically needed for DNA-binding. The histidines within the domain, together with the cysteines, bind to zinc ions, which is

required for the DNA-binding activity of the Zinc-finger proteins. The proline encoded by PRD-repeat also has unique characteristics, in which the nitrogen atom of the amino acid is incorporated into a ring, as a result, a proline residue disrupts the usual organization of the backbone of a polypeptide, causing a sharp transition in the direction of the chain (see Lewin,1990). All these characteristics of the repetitive proline-histidine implies that PRD-repeat encodes a special protein domain. Testing for expression of the PRD-repeat in *Rhodnius* ovarioles using the synthetic oligonucleotides were negative, so the identification of tissue specific expression of PRD-repeat in *Rhodnius* system would be very helpful to understand the possible function of PRD-repeat.

Another open question to be answered is whether or not the *bicoid* homologous sequence found in *Rhodnius* is expressed only in the ovariole germ cells or also in the surrounding somatic cells, because the isolated *Rhodnius* ovariole RNA is from maternal nurse cell-oocyte as well as somatical follicle cells which is also active in RNA synthesis (Vanderberg,1963). *In situ* hybridization would indicate where the *bicoid* homologous sequence is expressed in *Rhodnius* ovarioles. If it is expressed in nurse cells, it may be related to a maternal gene that plays a role in development and it would be worthwhile to clone the gene and explore how it is translocalized into the oocytes, the mechanisms involved and its possible functions. If it is expressed in the follicle cells, then it would be of interest to determine the spatial and temporal expression of the gene, because the follicle cells are not homogenous but to have different subpopulations

(Huebner, 1984). Attempts to do *in situ* localization thus far have been inconclusive. Positive labelling of ovariole sections have shown expression in all the tissue of germ cell and somatic cell origin. Controls have not been unequivocal. Efforts to resolve this are underway and continuing. Also, the research in *Drosophila* shows that the gene expression in the follicle cells are spatially and temporally restricted and affects the polarity of the whole embryo (St. Johnston and Nusslein-Volhard,1992). It would be interesting if the *bicoid* homologous sequence is expressed in *Rhodnius* ovariole follicle cells.

Overall, *vasa* and *bicoid* homologous sequences were detected in the *Rhodnius* genome and ovariole RNA. The subregion in the *bicoid* cDNA p1128 that is responsible for the hybridization was analysed and only the 252 bp fragment of the p1128 hybridized to the *Rhodnius* DNA and ovariole RNA. The PRD-repeat was found to be conserved in the *Rhodnius* genome, suggesting that PRD-repeat is a functionally important DNA motif.

LITERATURE CITED

Berleth,T.,Burri,M.,Thoma,G.,Bopp,D.,Richstein,S.,Frigerio,G.,Noll,M. and Nusslein-Volhard,C. 1988. The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. EMBO J. 7(6), 1749-1758.

Bopp,D.,Burri,M.,Baumgartner,S.,Frigerio,G.and Noll,M. 1986. Conservation of a large protein domain in the segmentation gene *paired* and in functionally related genes of *Drosophila*. Cell 47,1033-1040.

Bopp,D.,Jamet,E.,Baumgartner,S.,Burri,M. and Noll,M. 1989. Isolation of two tissue specific *Drosophila* paired box genes *Pox meso* and *Pox neuro*. EMBO.J. 8,1183-1190.

Buehr,M.L. and Blackler,A.W. 1970. Sterility and partial sterility in the south african clawed toad following pricking of the egg. J. Embryol.Exp.Morphol. 23,375-384.

Capco,D.G. and Jeffery,W.R. 1982. Transient localizations of messenger RNA in *Xenopus laevis* oocytes. Dev. Biol. 39,1-12.

Carpenter,C.D. and Klein,W.H. 1982. A gradient of poly(A)$^+$RNA sequences in *Xenopus laevis* eggs and embryos. Dev. Biol. 91,43-49.

Chirgwin,J.M.,Przybyla,A.E.,Macdonald,R.J. and Rutter,W.J. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18(24), 5294-5299.

Chomczynski,P. and Sacchi,N. 1987. Single-step method of isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry. 162,156-159.

Chung,H.M. and Malasinski,G.M. 1983. Reversal of developmental competence in inverted amphibian eggs. J. Embryol.Exp.Morphol. 73,207-220.

Darnell,J.,Lodish,H. and Baltimore,D. 1990. Molecular Cell Biology 2nd Ed. W.H. Freeman and Company, New York.

Diehl-Jones,W. and Huebner,E. 1992. Spatial and temporal transcellular current patterns during oogenesis. Dev. Biology 153,302-311.

Driever,W. and Nusslein-Volhard,C. 1988a. A gradient of *bicoid* protein in *Drosophila* embryo. Cell 54,83-93.

Driever,W. and Nusslein-Volhard,C. 1988b. The *bicoid* protein determines position in the *Drosophila* embryo in a concentration -dependent manner. Cell 54,83-93.

Driever,W. and Nusslein-Volhard,C. 1989a. The *bicoid* protein is a positive regulator of *hunchback* transcription in the early *Drosophila* embryo. Nature 337,138-143.

Driever,W. and Nusslein-Volhard,C. 1989b. Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity binding sites for the *bicoid* morphogen. Nature 340,363-367.

Ephrussi,A. Dickinson,L.K.,and Lehmann,R. 1991. *Oskar* organizes the germ plasm and directs localization of the posterior determinant *nanos*. Cell 66,37-50.

Ephrussi,A. and Lehmann,R. 1992. Induction of germ cell formation by *oskar*. Nature 358,387-392.

Frigerio,G.,Burri,M.,Bopp,D.,Baumgartner,S. and Noll,M. 1986. Structure of the segmentation gene *paired* and the *Drosophila* PRD gene set as part of a gene network. Cell 47,735-746.

Frohnhofer,H.G. and Nusslein-Volhard,C. 1986. Organization of anterior pattern in the *Drosophila* embryo by the maternal gene *bicoid*. Nature 324,120-125

Frohnhofer,H.G. and Nusslein-Volhard,C. 1987. Maternal genes required for the anterior localization of *bicoid* activity in the embryo of *Drosophila*. Genes & Development 1,880-890.

Gerhart,J.,Black,S.,Gimlich,R.,and Scharf,S. 1983. Control of polarity in amphibian egg. In Time ,Space and Embryonic Development. W.R.Jeffery and R.A.Raff eds. (New York: Alan R.Liss,Inc.) pp.261-286.

Gilbert,W. 1978. Why genes in pieces ? Nature 271,9.

Gilbert,W.,Marchionni,M. and McKnight,G. 1986. On the antiquity of introns. Cell 46,151-154.

Gurdon,J.B. Mohun,T.J., Fairman,S. and Brenan,S. 1985. All components required for the eventual activation of muscle-specific actin genes are localized in

the subequatorial region of an uncleaved egg. Proc.Natl.Acad.Sci. USA 82,139-143.

Hay,B.,Jan,L.Y. and Jan,Y.N. 1990. Localization of *vasa*, a component of *Drosophila* polar granules, in maternal-effect mutants that alter embryonic anteroposterior polarity. Development 109,425-433.

Huebner,E. and Anderson,E. 1972. A cytological study of the ovary of *Rhodnius prolixus* I. The ontogeny of the follicular epithelium. J. Morph. 136, 495-504.

Huebner,E. 1984a. The ultrastructure and development of the telotrophic ovary. In Insect Ultrastructure and Development. Vol.2. R.C.King and H.Akai (eds). Plenum Press, New York. pp3-48.

Huebner,E. 1984b. Developmental cell interactions in female insect reproductive organs. In Advances in invertebrate reproduction. Vol.3 W.Engels (eds) Elsevier Science Publishers B.V.,Amsterdam. pp.97-105.

Hulskamp,M.,Pfeifle,C. and Tautz,D. 1990. A morphogenetic gradient of *hunchback* protein organizes the expression of gap genes *Kruppel* and *Knirps* in the early *Drosophila* embryo. Nature 346, 577-580.

Jeffery,W.R. and Capco,D.G. 1978. Differential accumulation and localization of poly(A)$^+$-containing RNA during early ascidian development of the ascidian *Styela*. Dev. Biol. 67,152-166.

Jeffery,W.R.,Tomlinson,C.R. and Brodeur,R.D. 1983. Localization of actin messenger RNA in the cortex during early ascidian development. Dev. Biol. 99,408-417.

Jeffery,W.R. 1984. Spatial distribution of messenger RNA in the cytoskeletal frameworkof ascidian eggs. Dev. Biol. 103,482-492.

Jeffery,W.R. 1985. The spatial distribution of maternal mRNA is determined by a cortical cytoskeletal domain in *Chaetopterus* eggs. Dev.Biol. 110,217-229.

Jeffery,W.R. and Wilson,L. 1983. Localization of messenger RNA in the cortex of *Chaetopterus* eggs and early embryos. J.Embryol.Exp.Morphol. 4,320-326.

Kageura,H. and Yamana,K. 1984. Pattern regulation in defect embryos of *Xenopus laevis*. Dev. Biol. 101,410-415.

Kastern,W.H.,Watson,C.A. and Berry,S.J. 1990. Maternal messenger RNA

distribution in silkmoth eggs. I.Clone Ec4B is associated with the cortical cytoskeleton. Development. 108,497-505.

Kelly,G.M. 1989. Embryogenesis of the insect:*Rhodnius prolixus*, cellular, biochemical and molecular aspects. Ph.D. thesis. Dept. of Zoology Univ. of Manitoba.

Kilchherr,F.,Baumgartner,S.,Bopp,D.,Frei,E. and Noll,M. 1986. Isolation of the *paired* gene of *Drosophila* and its spatial expression during early embryogenesis. Nature 321,493-499.

Kim-Ha,J.,Smith,J.L.and Macdonald,P.M. 1991. *Oskar* mRNA is localized to the posterior pole of the *Drosophila* oocyte. Cell 66,23-35.

King,M.L. and Barklis,E. 1985. Regional distribution of maternal messenger RNA in the amphibian oocyte. Dev. Biol. 112,203-212.

King,R.C. 1970. Ovarian development in *Drosophila melanogaster*. New York, Academic Press.

Lasko,P.F. and Ashburner,M. 1988. The product of the *Drosophila* gene *vasa* is very similar to eukaryotic initiation factor-4A. Nature 335,611-617.

Lasko,P.F.and Ashburner,M. 1990. Posterior localization of *vasa* protein correlates with, but not sufficient for, pole cell development. Genes & Development 4,905-921.

Lehmann,R. and Nusslein-Volhard,C. 1986. Abdominal segmentation,pole cell formation and embryonic polarity require the localized activity of *oskar*, a maternal gene in *Drosophila*. Cell 47,141-152.

Lehmann,R. and Nusslein-Volhard,C. 1987a. *Hunchback*, a gene required for segmentation of an anterior and posteror region of the *Drosophila* embryo. Dev. Biol. 119,402-417.

Lehmann,R. and Nusslein-Volhard,C. 1987b. Involvement of the *pumilio* gene in the transport of an abdominal signal in the *Drosophila* embryo. Nature 329, 167-170.

Lehmann,R. and Nusslein-Volhard,C. 1991. The maternal gene *nanos* has a central role in the posterior pattern formation in the *Drosophila* embryo. Development 112,679-691.

Lewin,B. 1990. Genes IV. Oxford University Press. pp 8-10.

Macdonald,P.M. and Struhl,G. 1988. Cis-acting sequences responsible for anterior localization of *bicoid* mRNA in *Drosophila* embryos. Nature 336,595-598.

Macdonald,P.M. 1990. *Bicoid* mRNA localization signal: phylogenetic conservation of function and RNA secondary structure. Development 110,161-171.

McPherson,S. and Huebner,E. 1993. Dynamics of the oocyte cortical cytoskeleton during oogenesis in *Rhodnius prolixus*. Tissue and Cell 25(3),399-421.

McGinnis,W.,Levine,M.S.,Hafen,E.,Kuroiwa,A. and Gehring,W.J. 1984. A conserved DNA sequence in homeotic genes of the *Drosophila* antennapedia and bithorax complexes. Nature 308,428-433.

McGinnis,W.,Garber,R.,Wirz,J.,Kuroiwa,A. and Gehring,W.J. 1984. A homologous protein-coding sequence in *Drosophila* homeotic genes and its conservation in other metazoans. Cell 37,403-408.

Melton,D.A. 1987. Translocation of a localized maternal mRNA to the vegetal pole of *Xenopus* oocytes. Nature 328,80-82.

Mohler,J.,Eldon,E.D. and Pirrotta,V. 1989. A novel spatial transcription pattern associated with the segamentation gene, *giant*, of *Drosophila*. EMBO. J. 8,1539-1548.

Moon,R.T.,Nicosia,R.F.,Olsen,C.,Hille,M.B. and Jeffery,W.R. 1983. The cytoskeletal framework of Sea urchin eggs and embryos:developmental changes in the association of messenger RNA. Dev.Biol. 95,447-458.

Mosquera,L.,Forristall,C.,Zhou,Y. and King,M.L. 1993. A mRNA localized to the vegetal cortex of *Xenopus* oocytes encodes protein with a *nanos*-like zinc finger domain. Development 117, 377-386.

Mowry,K.L. and Melton,D.A. 1992. Vegetal messenger RNA localization directed by a 340-nt RNA sequence element in *Xenopus* oocytes. Science 255,991-994.

Nieuwkoop,P.D. 1977. Origin and establishment of embryonic polar axes in amphibian development. Curr. Top. Dev. Biol. 11,115-132.

Nusslein-Volhard,C.,Frohnhofer,H.G. and Lehmann,R. 1987. Determination of antero-posterior polarity in *Drosophila*. Science 238,1675-1681.

Nusslein-Volhard,C. 1991. Determination of the embryonic axes of *Drosophila*. Development (suppl) 1, 1-10.

Okada,M.,Kleinman,I.A. and Schneiderman,H.A. 1974. Restoration of fertility in sterilized *Drosophila* eggs by transplantation of polar cytoplasm. Dev. Biol. 37,43-54.

Paglia,L.M.,Berry,S.J. and Kastern,W.H. 1976. Messenger RNA synthesis, transport, and storage in silk moth ovarian follicles. Dev. Biol. 51,173-181.

Pankratz,M.J.,Hoch,M.,Seifert,E. and Jackle,H. 1989. *Krupple* requirement for *Knirps* enhancement reflects overlapping gap gene activities in the *Drosophila* embryo. Nature 341,337-340.

Phillips,C.R. 1982. The regional distribution of poly(A)$^+$ and total RNA concentrations during early *Xenopus* development. J.Exp.Zool. 223,265-275.

Pokrywka,J.N. and Stephenson,E. 1991. Microtubules mediate the localization of *bicoid* RNA during *Drosophila* oogenesis. Development 113,55-66.

Rebagliati,M.R.,Weeks,D.L.,Harvey,R.P. and Melton,D.A. 1985. Identification and cloning of localized maternal RNAs from Xenopus eggs. Cell 42,769 -777.

Rodgers,R.E. and Gross,P.R. 1978. Inhomogeneous distribution of egg RNA sequences in the early embryo. Cell 14,279-288.

Sambrook,J.,Fritsch,E.F. and Maniatis,T. 1989. Molecular Cloning. Cold Spring Harbor Laborory Press. New York.

Showman,R.M.,Wells,D.E.,Anstrom,J.,Hursh,D.A. and Raff,R.A. 1982. Messenger-specific sequestration of maternal histone mRNA in the *Sea urchin* egg. Proc.Natl.Acad.Sci.USA 79,5944-5949.

Schroder,C.,Tautz,D.,Seifert,E. and Jackle,H. 1988. Differential regulation of the two transcripts from the *Drosophila* gap segmentation gene *hunchback*. EMBO.J. 7,2881-2887.

Scott,M.P.,Tamkun,J.W.,and Hartzell,G.W. 1989. The structure and function of the homeodomain. Biochimica et Biophysica Acta. 989,25-48.

Smith,L.D. 1966. The role of a "germinal plasm" in the formation of premordial germ cells in *Rana pipiens*. Dev. Biol. 14,330-347.

Sommer,R.J.,Retzlaff,M.,Goerlich,K.,Sander,K. and Tautz,D. 1992. Evolutionary conservation pattern of zinc-finger domains of *Drosophila* segmentation

genes. Proc.Natl.Acad.Sci.USA. 89,10782-10786.

Steward,R.,and Nusslein-Volhard,C. 1986. The genetics of the dorsal-Bicaudal-D region of *Drosophila melanogaster*. Genetics 113,665 - 678.

St. Johnston,D.,Driever,W.,Berleth,T.,Richstein,S. and Nusslein-Volhard,C. 1989. Multiple steps in the localization of *bicoid* RNA to the anterior pole of the *Drosophila* oocyte. Development (Suppl.) 107,13-19.

St. Johnston,D.,Beuchle,D.,and Nusslein-Volhard,C. 1991. *Staufen*, a gene required to localize maternal RNA's in the *Drosophila* egg. Cell 66,51-63.

St. Johnston,D.and Nusslein-Volhard,C. 1992. The origin of pattern and polarity in the *Drosophila* embryo. Cell 68,201-219.

Struhl,G. 1989. Differing strategies for organizing anterior and posterior body pattern in *Drosophila* embryos. Nature 338,741-744.

Struhl,G.,Struhl,K. and Macdonald,P.M. 1989. The gradient morphogen *bicoid* is a concentration-dependent transcriptional activator. Cell 1259-1273.

Sudhof,T.C.,Russell,D.W.,Goldstein,J.L.,Brown,M.S.,Sanchez-Pescador,R. and Bell,G.I. 1985. Cassette of eight exons shared by genes for LDL receptor and EGF precursor. Science 228,893-895.

Suter,B.,Romberg,L.M. and Steward,R. 1989. Bicaudal-D, a *Drosophila* gene involved in developmental asymmetry:localized transcript accumulation in ovaries and sequence similarity to myosin heavy chain tail domains. Genes & Development. 3,1957-1968.

Suter,B. and Steward,R. 1991. Requirement for phosphorylation and localization of the Bicaudal-D protein in *Drosophila* oocyte differentiation. Cell 67,917-926.

Tautz,D.,Lehmann,R.,Schnurch,H.,Schuh,R.,Seifert,E.,Kienlin,A.,Jones,K. and Jackle,H. 1987. Finger protein of novel structure encoded by *hunchback*, a second member of the gap class of *Drosophila* segamentation genes. Nature 327, 383-389.

Theurkauf,W.,Smiley,S.,Wong,M.L. and Alberts,B.M. 1992. Reorganization of the cytoskeleton during *Drosophila* oogenesis: implications for axis specification and intercellular transport. Development 115,923-936.

Treisman,J.,Harris,E. and Desplan,C. 1991. The paired box encodes a second DNA-binding domain in the Paired homeo domain protein. Genes & Development 5,594-604.

Treisman,J.,Harris,E.,Wilson,D.and Desplan,C. 1992. The homeodomain: a new face for the helix-turn-helix ? Bioessays 14(3),145-150.

Vale,R.D. 1987. Intracellular transport using microtubule-based motors. Ann.Rev.Cell Biol. 3,347-378.

Vanderberg,J. 1963. Synthesis and transfer of DNA and RNA and protein during vitellogenesis in *Rhodnius prolixus* (Hemiptera). Bio. Bull. 125,556-575.

Venezsky,D.L.,Angerer,L.M. and Angerer,R.C. 1981. Accumulation of histone repeat transcripts in the *sea urchin* egg pronucleus. Cell 24,385-391.

Wahl,G.M.,Berger,S.L. and Kimmel,A.R. 1987. Molecular hybridization of immobilized nucleiz acids: theoretical concepts and practical considerations. In Methods in Enzymology. Vol 152. Berger,S.L. and Kimmel,A.R. eds. Academic Press,Inc. London.

Wang,C.and Lehmann,R. 1991. *Nanos* is the localized posterior determinant in *Drosophila*. Cell 66,637-647.

Wharton,R.P. and Struhl,G. 1989. Structure of the *Drosophila Bicaudal-D* protein and its role in localizing the posterior determinant *nanos*. Cell 59,881-892.

Wharton,R.P. and Struhl,G. 1991. RNA regulatory elements mediate control of *Drosophila* body pattern by the posterior morphogen *nanos*. Cell 67, 955-967.

Weeks,D.L. and Melton,D.A. 1987. A maternal mRNA localized to the vegetal hemisphere in *Xenopus* eggs codes for a growth factor related to TGF-β. Cell 51,861-867.

Wilson, E.B. 1928. "The Cell in Development and Heredity" 3rd ed. Macmillan Co. New York.

Yisraeli,J.K.,Sokol,S. and Melton,D.A. 1989. The process of localizing a maternal messenger RNA in *Xenopus* oocytes. In The Molecular Basis of Positional Signalling. R.Kay and J.Smith. eds. The Company of Biologists Ltd. Cambridge. pp 31-36.

Yisraeli,J.K. and Melton,D.A. 1988. The maternal mRNA Vg1 is correctly localized following injection into *Xenopus* oocytes. Nature 336,592-595.

Yisraeli,J.K.,Sokol,S. and Melton,D.A. 1990. A two-step model for the localization of maternal mRNA in *Xenopus* oocytes: involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 mRNA. Development 108,289-298.

Zhang,C.,Muller,J.,Hoch,M., Jackle,H. and Bienz,M. 1991. Target sequences for hunchback in a control region conferring ultrabithorax expression boundaries. Development 113,1171-1179