

Journal of Cell and Animal Biology

Volume 8 Number 6, June 2014

ISSN 1996-0867



*Academic
Journals*

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ARTICLES

How to obtain the organelles of prokaryotic and microbial eukaryotic cells

B. I. Aderiye and O. A. Oluwole

A note on interaction and pre-implantation development stages

Justin R. Chimka and Leiyang Jiang

Review

How to obtain the organelles of prokaryotic and microbial eukaryotic cells

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Received 23 May, 2014; Accepted 25 June, 2014

An organelle is a specialized functional subunit within cells carrying out specific functions. These compartments which may or may not be enclosed in a lipid bilayer are found in microorganisms. While those found in eukaryotic cells are usually enclosed in lipid bilayer, those in prokaryotes don't. All microbes have compartments common to them like the nucleic acids, protein, ribosomes as well as unique intracellular structures found only in microbial subgroups. Such compartments include the mitochondria, endoplasmic reticulum, golgi apparatus amongst others unique to all eukaryotic cells only. Prokaryotes contain some micro-compartments unique to them including the carboxysomes, lipid bodies, polyhydroxybutyrate granules. The right choice of cell disruption methods that limit damage to the compartments is important in achieving successful compartment isolation and purification. Commonly applied methods include sonication, enzymatic lysis, detergent lysis, cavitation amongst others depending on the type of cells involved. Fractionation is the commonly utilized method for isolation and purification of organelles, utilizing ultracentrifugation and techniques that exploits size, density and surface charge variations of protoplasmic content. Such techniques include gradient centrifugation methods, use of beads, affinity purification chromatography methods and electrophoresis. Here, we review the compartments in microbial cells and the techniques employed to isolate and purify these intracellular components.

Key words: cell disruption, purification, prokaryote, eukaryote, functional unit.

INTRODUCTION

The tree of life is made up of three distinct domains: eukaryotes, bacteria, and archaea (Forterre, 2001). The eukaryotes are organisms with cells organized into complex structures enclosed within defined membranes. These membrane-bound structures for example the nucleus, which contains the cells genetic materials distinguish them from other forms of life. Eukaryotes include animals, plants, fungi, and protozoa. The protozoa group includes an enormous number of species, comprising some agents of human and animal diseases such as malaria, and leishmaniasis (de Souza and da

Cunha-e-Silva, 2003). Most fungi are filamentous, many grow as unicellular yeasts (*Saccharomyces cerevisiae*) while some for example chytridomycetes grow as individual rounded cells or dichotomous branched chains of cells with root-like rhizoids for attachment to a nutrient resource (Kavanagh, 2011). In contrast, archaea and bacteria are prokaryotes and have no-known distinct cell nucleus or any other membrane bound organelles within their cells.

A typical microbial cell contains different types of intracellular membrane bound structures known as

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“compartments”. An organelle is a specialized functional subunit within a cell that has a specific function and usually separately enclosed within its own lipid bilayer. There are many types of organelles, particularly in eukaryotic cells. Most prokaryotes do not possess organelles per se, but members have protein-based micro-compartments (Yeates et al., 2008) which are conditionally expressed and are responsible for several metabolic processes (Cheng et al., 2008) They are thought to act as primitive compartments (Kerfeld et al., 2005). Micro-compartments are very large, structurally sophisticated, usually about 100 to 150 nm in view and consist of 10,000 to 20,000 polypeptides of 10 to 20 types (Cheng et al., 2008).

Eukaryotic cells are organized into separate membrane-bound compartments or compartments that perform vital biochemical reactions, with a specialized function and unique protein and lipid composition whereas their prokaryotic counterparts generally lack such sophisticated sub-specialization of the cytoplasmic space (Murat et al., 2010). However, decades of research have shown that a number of unique and diverse compartments can be found in the prokaryotic world raising the possibility that the ability to form compartments may have existed before the divergence of eukaryotes from prokaryotes (Shively, 2006; Murat et al., 2010).

All Eukaryotic cells have the same basic set of membrane-enclosed organelles (Alberts et al., 2002). The major intracellular compartments common to eukaryotic cells includes the nucleus housing the main genome and is the principal site of DNA and RNA synthesis. Also, the surrounding cytoplasm consists of the cytosol and the cytoplasmic organelles suspended in it. The cytosol, makes up a little more than half the total capacity of the cell; is the location of protein synthesis and degradation. It also performs most of the cell's intermediary metabolism—that is catabolic and anabolic processes (Alberts et al., 2002).

Prokaryotic organelles can be generally divided into two major groups based on the composition of the membrane layer surrounding them. First are the cellular structures bound by a non-unit membrane such as a protein shell or a lipid monolayer examples of which include lipid bodies, polyhydroxybutyrate granules, carboxysomes, and gas vacuoles (Shively, 2006). The other consists of those compartments that are surrounded by a lipid-bilayer membrane, an arrangement that is indicative of the canonical compartments of the eukaryotic endomembrane system (Murat et al., 2010).

The protozoa contain unique cytoplasmic structures and organelles (de-Souza and da-Cunha-e-Silva, 2003). These compartments are similar to those of other eukaryotic cells. The plasma membrane enclosing the cytoplasm also covers the projecting locomotory structures like pseudopodia, cilia, and flagella. Other compartments found in this group of protists are nucleus, cytosome (not all species), microtubules, golgi apparatus,

mitochondria, lysosomes, food vacuoles, conoids in Apicomplexa (Baron, 1996).

The cell envelope in yeasts and fungi is the peripheral structure that encases the cytoplasm and comprises the plasma membrane, the periplasm, the cell wall and additional extracellular structural components such as fimbriae and capsules). Their compartments are bathed in an aqueous cytoplasm containing soluble proteins and other macromolecules together with low-molecular-weight metabolites, although the hyphae of central and therefore older colony regions of filamentous fungi may become devoid of protoplasm, as it is driven forward with the growing tip (Kavanagh, 2011).

Cytoplasmic components additionally comprise microbodies, ribosomes, proteasomes, lipid particles and a cytoskeletal network. The latter confers structural stability to the fungal cytoplasm and consists of microtubules and microfilaments. The following membrane-bound compartments may be found in a typical fungal cell: nucleus (DNA and RNA), endoplasmic reticulum (ER), mitochondria, Golgi apparatus, secretory vesicles and vacuoles (Kavanagh, 2011). Several of these compartments form extended membranous systems. For example, the ER is contiguous with the nuclear membrane and secretion of fungal proteins involves inter-membrane trafficking in which the ER, Golgi apparatus, plasma membrane and vesicles all participate (Kavanagh, 2011). This paper examines the structure of some compartments in microbial cells and the method of isolation and purification of these structures.

ISOLATION AND PURIFICATION OF COMPARTMENTS FROM MICROBIAL CELLS

Cell disruption

Isolation of intracellular products and micro-compartments of microbial cells usually require a cascade of operations. This task usually starts from a cell disruption process. Disintegration of cells precedes the fractionating and purification of constituent of the cell structures and influences the quality of the final product (Savov et al., 2001). Various cell disruption methods have been developed to establish an efficient, low-cost, and effective release of intracellular contents (Middelberg, 1995; Geciova et al., 2002; Ho et al., 2008). Disruption can be achieved by procedures such as exposure to high-frequency sound (sonication) (Ghorbanzadeh-Mashkani et al., 2013), treatment with a high-speed blender (Bodzon-Kulakowska et al., 2007), grinding in a mechanical homogenizer (Van Het Hof et al., 2000), bead milling (Doucha and Livansky, 2008) as well as nitrogen cavitation (Gottlieb and Adachi, 2000). Antibiotics, chelating agents, detergents, and solvents are also capable of disintegrating cells (Leuko et al., 2008; Doolan and Wilkinson, 2009) Klimek-Ochab et al., (2011) carried

out a comparative study on different methods for disrupting the cell wall of fungi. Several techniques utilized included ultrasound disintegration, homogenization in bead mill, application of chemicals of various types, and osmotic shock. Usually, it is necessary to monitor the process by phase contrast microscopy in order to avoid rupture of the compartments or disorganization of cytoplasmic structures (de Souza and Cunha da Silva, 2003).

Cell fractionation

The cell fractionation technique is the most commonly used technique. Once the cells are broken open, the suspension can be separated into its main components using a series of centrifugations at increasing speeds. This causes cell components to move toward the bottom of the centrifuge tube, forming a pellet at a rate that depends on their size and density. The supernatant is then collected and subjected to further centrifugation at higher speed, and the process may be repeated several times depending on the cell type and the structures to be isolated. Usually differential centrifugation does not yield very pure material. Therefore, it is necessary to continue the isolation procedure by using density-gradient centrifugation, a procedure where the compartments and structures are separated by sedimentation through a gradient of a dense solution such as sucrose, Metrizamide, Percoll (de Souza and Cunha da Silva, 2003).

FUNCTIONAL UNITS AND COMPARTMENTS FOUND IN ALL MICROBIAL CELLS

The following are some of the compartments found in most microbial cells:

Flagellum

The flagellum is responsible for the motility of motile organisms and participates in their attachment and initiating the contact with mammalian cells (De Souza, 1984), and play important role in infection development (Duan et al., 2013). Bacteria flagella are dynamic, helical filaments with different arrangements viz monotrichous (*Vibrio cholerae*), lophotrichous, amphitrichous and peritrichous arrangement found in *Escherichia coli* (Manson, 2010). Eukaryotic flagella have the 9+2 structural arrangement with internal fibrils. Montie et al. (1981) isolated and purified flagella from strains of *Pseudomonas aeruginosa* were isolated by shearing the flagella before using differential centrifugation procedure to extract the typical filament. They observed that the isolation of highly purified, single banded flagellin could be accomplished by elution of the 53000 molecular weight band. Also, Ibrahim et al. (1985) isolated highly

purified flagellin from different Salmonella serotypes, and the cells were pelleted by centrifugation before flagella were detached by exposure to low pH (2). The flagellin was purified by ultracentrifugation, NH_2SO_4 precipitation and dialysis before purity was confirmed via SDS-PAGE. Segura et al. (1977) disrupted *T. cruzi* epimastigotes using cavitation. The flagellum-enriched fraction obtained by differential centrifugation was contaminated with membranes of flagellar and non-flagellar origins, due to the cell rupture method. The researchers performed double diffusion tests against an antibody raised to the entire parasite and found five precipitin lines against the flagellar fraction. Flagella also showed good results (80 to 90%) in protective studies against a lethal challenge with trypomastigotes. The use of adjuvants raised this protection to 100% (Ruiz et al., 1986).

Pereira et al. (1977) designed another method for isolating flagella based on separating the flagellum from the undisrupted cell body. They treated *Crithidia fasciculata*, *Herpetomonas samuelpessoai* and *Leishmania tarentolae* with Lubrol-PX, a non-ionic detergent, in the presence of magnesium chloride, before deflagellation with a Dounce homogenizer. Flagellar fractions thus obtained were very pure, but consisted of demembrated flagella as a result of the detergent. Although devoid of membranes, isolated flagella from *H. samuelpessoai* were very effective in protecting mice against *T. cruzi*, indicating that highly conserved antigens were present inside the flagella.

The best way of investigating the paraflagellar rod composition of *H. megaseliae* was to obtain a subcellular fraction containing purified and intact paraxial rods. Russell et al. (1983) used trypsin to disrupt the connections between paraxial rod and axoneme. Purified and detergent demembrated flagella from *H. megaseliae* were treated with trypsin in a very careful manner and applied on top of a 1.8-2.2 M continuous sucrose gradient. After centrifugation, six fractions of equal volume were collected from the top to bottom. The third fraction was a highly purified para-flagellar rod fraction (de Souza and Cunha-e-Silva, 2003). Cunha-e-Silva et al. (1989) managed to get a highly purified flagellar membrane fraction from *H. megaseliae* and *T. cruzi* epimastigotes by combining detergent treatment on ice, vortexing and separation on a sucrose equilibrium gradient. Analyzing the purified fraction by using filipin cytochemistry for sterols combined with freeze-fracture showed that the flagellar membrane is almost devoid of intramembranous particles and rich in sterols (Cunha-e-Silva et al., 1989).

Plasma membrane

Isolation of highly purified plasma membranes is crucial in its proteomics (Lee et al., 2012). The plasma membrane is dynamic, with both its lipid and protein composition changing to facilitate adaptation to the ambient

conditions (Xiao, 2006). Plasma membranes can be prepared from yeast by initially spheroplasting the cells (Chaney and Jacobsen, 1983). Though these procedures give high yields, an extended incubation (at least 30-45 min at 30°C) with zymolyase is required in order to remove the cell walls. This process however will almost certainly cause physiological changes in the yeast cells, which may be reflected in alterations to plasma membrane components and will certainly influence biochemical activities of plasma membrane proteins as well as the MAP kinase cascade that responds to cell integrity (Reinoso-Martin et al., 2003). Also, cells cannot be spheroplasted in certain physiological states, such as stationary phase. Spheroplasting is therefore unsuitable because of the effects of stress on the proteins of the *S. cerevisiae* plasma membrane (Panaretou and Piper, 1992).

Rapidly disrupting cells by vortexing with glass bead in which membranes are banded on sucrose density gradients avoids this problem (Serrano et al., 1991; Xiao, 2006). The plasma membranes obtained are of high purity, and the procedure is ideally suited to comparative studies of the plasma membranes from cells of different physiological states. One of the best ways to assess the purity of yeast plasma membranes is to assay the fraction of the ATPase activity subject to orthovanadate inhibition (Serrano, 1988). The plasma membrane ATPase is inhibited by orthovanadate.

Yields from the glass bead method tend to be low, but the membranes obtained are of high purity. If high yields are required, spheroplasted cells can be disrupted and membranes can be isolated via entrapment by dense cationic silica beads (Chaney and Jacobsen, 1983). It should be noted that treatment with enzymes that degrade the cell wall could change biochemical properties of membrane proteins as well as affect levels of the proteins themselves.

Hullenga et al. (1994) isolated the functional plasma membranes from the filamentous fungus *Penicillium chrysogenum* with the objective of studying transport processes. The isolation procedure from whole cells instead of protoplasts involved three steps, viz. homogenization of cells with a Braun MSK homogenizer, followed by Percoll gradient centrifugation and floatation of membranes in a three-step Nycodenz gradient. Purity was ascertained via cytochemical staining with phosphotungstic acid.

The continuous layer of microtubules below the plasma membrane makes trypanosomatids resistant to conventional methods for cell breakage. Hunt and Ellar (1974) disrupted *Leptomonas collosoma* using glass beads and were able to obtain a plasma membrane fraction. To obtain a purified plasma membrane fraction from epimastigotes of *T. cruzi*, cells previously swollen by treatment with Triton X-100 were disrupted using a Dounce-type homogenizer followed by consecutive steps of differential centrifugation and its purity was evaluated

by electron microscopy and by testing for enzyme markers. Electron-microscopy showed slight contamination with ribosomes of *T. cruzi* (Timm et al., 1980). In another approach, cells were ruptured by sonication and the membrane fraction was isolated by differential centrifugation, followed by equilibrium centrifugation on sucrose gradients.

To obtain a plasma membrane fraction from *T. brucei*, cells were disrupted using nitrogen cavitation, sonication or a dounce homogenizer followed by differential centrifugation and successive centrifugations in gradients of dextran and sucrose (Rovis and Baekkeskov, 1980). The fraction obtained was characterized by electron microscopy and assay of the enzyme markers $\text{Na}^+\text{-K}^+$ ATPase and adenyl cyclase (Voorheis et al., 1979).

In another method, plasma membrane was induced to vesiculate by incubating the cells with aldehydes, N-ethylmaleimide, p-chloromercuribenzoate, or acid buffers, followed by isolation of the vesicles by sucrose-density centrifugation (da Silveira et al., 1979). Examination of the electron micrographs of *T. cruzi* treated with vesiculating agents showed that vesicles form only at certain points of the membrane which encloses the cell body and the flagellum. Therefore, the membrane fraction isolated by this procedure certainly does not contain all areas of the plasma membrane. The method shows that some specific regions of the cell surface of *T. cruzi* that are more likely to form vesicles.

Ribosomes

Cell disruption is first done employing either enzymatic lysis, or sonication. The ribosomes are prepared by centrifugation (Spedding, 1990). After removal of unbroken cells by centrifugation, the supernatant was centrifuged to pellet the ribosomes. For *S. pneumoniae*, cells are first microfluidized using sufficient cycles to produce at least 50% cell disruption. The lysed cells are then spun at 4°C, followed by centrifugation to pellet the ribosomes. French -pressing is done coupled with addition of RNase free DNase before loading into a column of cysteine Sulfolink resin. The column is washed with lysis buffer and then eluted with the same buffer either by isocratic elution or with a gradient from 0 to 100% elution buffer. The flow rates for washing and elution is set by the maximum pressure limit for the resin (0.15 MPa). Ribosomes are pelleted from pooled fractions and resuspended in buffer for snap freezing in liquid nitrogen and storage at -80°C.

Early method of purification of ribosomes was by ultracentrifugation. However, three new methods have been advanced; affinity purification using ribosomally targeted antibiotics (for example, chloramphenicol and erythromycin) as bait (Le Goffic et al., 1974, 1980), size exclusion chromatography (SEC) (Jelenc 1980), and hydrophobic interaction chromatography (HIC) (Kirillov et

al., 1978; Fabry et al., 1981; Saruyama, 1986). There is need of a method that ensures rapid isolation of active ribosomes from bacteria without the use of harsh conditions or lengthy procedures that damage ribosomes. Maguire et al. (2008) developed a novel chromatography method that ensures that ribosomes obtained are intact and active when extracted from *Escherichia coli*, *Deinococcus radiodurans*, and some clinical isolates of *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA). The system employs cysteine Sulfolink resin and shows unique features compared to other chromatography methods used for ribosomes or RNA.

The utility of cysteine-Sulfolink chromatography has been clearly demonstrated with ribosomes from four very different species. Both 30S and 50S subunits, as well as 70S monosomes and polysomes all bind and elute from the resin. The ability to reduce degradation of ribosomes is likely a matter of speed, where the proteases and nucleases, which are present at very high levels in clinical isolates, start to separate from the ribosomes as soon as the latter bind to the resin. Sucrose-density gradient centrifugation serves well as a secondary purification to separate ribosomal subunits, monosomes, and polysomes, but gel filtration could also be considered (Maguire et al., 2008). Other methods such as parallel ribosome purification from multiple strains and purification of other ribonucleoprotein complexes or nucleic acids can also be employed.

Nucleic acids (DNA and RNA)

The extraction of DNA, RNA, and protein biomolecules, is the most crucial method used in molecular biology. DNA, RNA, and protein can be isolated from any biological material such as living or conserved tissues, cells, virus particles, or other samples for analytical or preparative purposes (Wink, 2006). Two types of DNA that can be isolated, purified and analysed include the recombinant DNA constructs such as plasmids or bacteriophage and chromosomal or genomic DNA from prokaryotic or eukaryotic organisms (Tan and Yiap, 2009; Odeyemi et al., 2014).

To successfully purify nucleic acid four important steps are generally required: effective disruption of cells or tissue; denaturation of nucleoprotein complexes; inactivation of nucleases (RNase for RNA extraction and DNase for DNA extraction), avoiding contamination from external sources (Doyle, 1999; Tan and Yiap, 2009). Contaminants to be avoided when extracting nucleic acids includes protein, carbohydrate, lipids, or other nucleic acid, for example, DNA free of RNA or RNA free of DNA (Buckingham and Flaws, 2007). The quality and integrity of the isolated nucleic acid will directly affect the results of all succeeding scientific research (Cseke et al., 2004).

RNA is an unsteady molecule and has a very short half-life once extracted from the cell or tissues (Brooks, 1998). There are several types of naturally occurring RNA including ribosomal RNA (rRNA) (80 to 90%), messenger RNA (mRNA) (2.5 to 5%) and transfer RNA (tRNA) (Buckingham and Flaws, 2007). Special care and precautions are required for RNA isolation as it is prone to degradation (Kojima and Ozawa, 2002). RNA is especially unstable due to the ubiquitous presence of RNases which are enzymes present in blood, all tissues, as well as most bacteria and fungi in the environment (Brooks, 1998; Buckingham and Flaws, 2007).

Strong denaturants have always been used in intact RNA isolation to inhibit endogenous RNases (Doyle, 1999). RNase is heat-stable and refolds following heat denaturation. The most common isolation methods can be divided into two classes: utilization of 4 M guanidinium thiocyanate and utilization of phenol and SDS (Doyle, 1999). Organic solvent-phenol-chloroform extraction is an example which is widely used in isolating nucleic acid from samples before electrophoresis on a Novex 6% acrylamide TBE/urea gel e.g. Invitrogen, Inc. for 100 min at 180V (Maguire et al., 2008). The electrophoresed gels are then stained with ethidium bromide for detection of RNA by fluorescence.

The main problem of any extensive DNA purification is that multi-step procedures reduce the yield of DNA, therefore compelling to process large amounts of biomass, thus increasing costs and time required (Cardinali et al., 2001). Conversely, small scale, rapid methods based on partial purification of DNA have a much higher efficiency of extraction, though often yielding DNA samples contaminated with protein or RNA. Even though the level of purification of the above methods is apparently compatible with many molecular applications, the presence of residual proteins, especially RNA, heavily affects the spectrophotometric reading and consequently the calculation of DNA concentration (Cardinali et al., 2001).

Alkaline extraction method for DNA

Alkaline lysis has been used to isolate plasmid DNA in *Escherichia coli* (Sambrook and Russell, 2001). It works well with all strains of *E. coli* and with bacterial cultures in the presence of Sodium Dodecyl Sulfate (SDS). The principle of the method is based on selective alkaline denaturation of high molecular weight chromosomal DNA while covalently closed circular DNA remains double stranded (Birnboim and Doly, 1979). Bacterial proteins, broken cell walls, and denatured chromosomal DNA become enmeshed into large complexes that are coated with sodium dodecyl sulfate. Plasmid DNA can be recovered from the supernatant after the denatured material has been removed by centrifugation.

The solid-phase nucleic acid purification which is readily

available as commercial extraction kits permits quick and efficient purification of nucleic acids as compared to orthodox techniques (Esser et al., 2005). Many of the hitches encountered with liquid-liquid extraction such as incomplete phase separation can be avoided. The solid phase system absorbs nucleic acid in the extraction process based on the pH and salt content of the buffer. Four crucial steps involved in solid-phase extraction are cell lysis, nucleic acids adsorption, washing, and elution (Kojima and Ozawa, 2002). Solid-phase purification is generally performed by using a spin column, operated under centrifugal force (Gjerse et al., 2009). Silica matrices, glass particles, diatomaceous earth, and anion-exchange carriers are examples of techniques previously employed in solid-phase extraction as the solid support.

Magnetic bead based nucleic acid purification

Magnetic separation is a simple and efficient way which is used in purification of nucleic acid nowadays. Many magnetic carriers are now commercially available. Particles having a magnetic charge may be removed by using a permanent magnet in the application of a magnetic field. Often, magnetic carriers with immobilized affinity ligands or prepared from biopolymer showing affinity to the target nucleic acid are used for the isolation process. Particles having magnetic or paramagnetic properties are employed in an invention where they are encapsulated in a polymer such as magnetizable cellulose (Nargessi, 2005). In the presence of certain concentrations of salt and polyalkylene glycol, magnetizable cellulose can bind to nucleic acids. Small nucleic acid requires higher salt concentrations for strong binding to the magnetizable cellulose particles (Tan and Yiap, 2009).

Another extraction kit has the same principle as the extraction described above, which used the magnetic-particle technology for nucleic acid purification (QIAGEN, 2008). It combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. Nucleic acid purification by using zirconia bead is another type of magnetic bead based purification. These microspherical paramagnetic beads have large available binding surface and can be dispersed in solution. This characteristic allows thorough nucleic acid binding, washing, and elution. The total nucleic acid isolation kit, which uses this technology for the nucleic acid purification, makes use of the mechanical disruption of samples with zirconia beads in a guanidinium thiocyanate-based solution that not only releases nucleic acid but also inactivate nuclease in the sample matrix (Applied Biosystems, 2008). Paramagnetic beads are added to the samples for the nucleic acid binding purpose. The mixture of beads and nucleic acid are immobilized on magnets and washed to remove protein and contaminants. Removal of residual binding

solution is done with a second wash solution and finally the nucleic acid is eluted in low-salt buffer (Applied Biosystems, 2008).

Extraction and purification of nucleic acids from viruses

For genomic and metagenomic analyses, prefiltration of the sample from which viruses will be harvested is desirable, involving the use of a 0.2- μm filter to remove prokaryotic and eukaryotic cells hence ensuring that the majority of the nucleic acid extracted from the sample will be viral (Steward and Culley, 2010). However, loss of virus may occur from this step (Paul et al., 1991). Fractionation in buoyant density gradients is an alternative to 0.2 μm filtration for separating cells and viruses (Lawrence and Steward 2010), since most viruses are more dense than most cells but the separation will not be absolute, because the density ranges of cells and viruses overlap. Tangential flow filtration (TFF) using an ultrafiltration membrane (typically 30,000 to 100,000 nominal molecular weight cutoff) is the method mostly employed to harvest viral assemblages from natural water samples (Steward and Culley, 2010).

The most common methods used to release nucleic acids from virions involve the use of heat, osmotic shock, detergents, chaotropic salts, or organic solvents, either alone or in combination, all of which lead to denaturation of capsid proteins (Ralph and Bergquist 1967). Tris buffer is used to prevent chemical hydrolysis of the nucleic acids. However, the simplest method by far to release nucleic acids from virions is to heat the sample (typically to 45 to 100°C). This alone is sufficient for some applications (Richardson et al., 1988), in particular for obtaining nucleic acids from purified viruses where nuclease contamination is expected to be minimal.

Osmotic shock can be used to disintegrate the capsids of some viruses, but others are resistant to this treatment (Anderson, 1950; Anderson et al., 1953). Formamide will also disrupt phage capsids and has been used as a rapid, simple, but perhaps less effective (Sambrook and Russell 2001), alternative to treatment with heat, SDS, and proteinase K digestion for extraction of DNA from viruses (Vega Thurber et al., 2009). This phenomenon may therefore facilitate some extraction protocols, but is generally not relied on. Also, the use of detergent for example, SDS when employed, solubilizes the capsids (Reynolds and Tanford, 1970) and may be used alone or with heat (Steward and Culley, 2010).

In separating nucleic acids from other macromolecules, it may be necessary to separate the nucleic acids from other macromolecules in the lysate. This may be achieved by exploiting differences in solubility or buoyant density among macromolecules. Five general approaches to this task are:

- 1) Organic extraction ((Kirby 1957),

- 2) Differential precipitation (Steward et al., 1992),
- 3) Solid-phase extraction (Boom et al., 1990),
- 4) Density gradient fractionation (Rickwood 1989), and
- 5) Electrophoresis (Marziali et al., 2005).

Commercial extraction/purification kits or reagents

Commercial purification kits or reagents are available that rely on the extraction principles outlined above of selective precipitation (for example, MasterPure™, Epicenter; Genra® Puregene®, Qiagen), selective adsorption (example, UltraClean®, Microbial DNA isolation kit, Mo Bio Laboratories; QIAamp®, MinElute®, Qiagen; AllPrep®, Qiagen) or selective solubility (for example, TRI Reagent®, Molecular Research Center; TRIzol®, Invitrogen). The nature of the starting material and final purity of the nucleic acids required will vary widely depending on the application hence; any of the methods above can be successfully applied for extraction and purification of nucleic acids in viruses.

Isolation of nucleic acids from yeasts

One of the challenges of isolating nucleic acids from yeast cells is the cell wall. The two main methods of overcoming this barrier are first to create spheroplasts and isolate from them (Cryer et al., 1975), or to use vortexing and glass beads to break through the cell wall (Hoffman and Winston, 1987; Xiao, 2006). The use of glass beads is better as it is quick and straightforward, and eliminates the expense of using zymolyase. The DNA isolated by this method is suitable for restriction digest and Southern blots, or for transformation into *E. coli*. Several methods for the extraction of yeast genomic DNA have been developed (Smith and Halvorson, 1967; Holm et al., 1986; Philippsen et al., 1991; Varma and Kwon-Chung, 1991; Mathaba et al., 1993; Min et al., 1995).

These protocols differ in several aspects such as extent of extraction (amount of biomass processed), efficacy with different yeast species, concentration of protein or RNA contaminants in the final solution, integrity of DNA (average length) and time required. The method used to isolate RNA is straightforward; the difficulty usually encountered in working with RNA is contamination by exogenous ribonucleases (RNases). Isolating genomic DNA involves vortexing for 3 min as compared to plasmid DNA which requires 2 min (Xiao, 2006). To purify extracted DNA, RNases are added to degrade contaminating RNA.

Protein

The first step in protein purification is cell lysis. In order to purify and analyze protein efficiently, they must be first released from their host cell in a soluble form. Protein

extraction from fungi and bacteria appears more challenging than mammalian cells due to their stable cell wall that is stronger than the plasma membrane (Tan and Yiap, 2009). Cell-based extraction is a critical step for almost all protein purification. Protein can be extracted by a few methods such as detergent lysis, shearing force, treatment with low ionic salt (salting out), and rapid changes in pressure, which aimed to weaken and break the membranes surrounding the cell to allow proteins to escape (Watson et al., 2004). Mechanical disruption techniques, such as French Press or glass beads are usually used to remove the cell wall, followed by detergent based extraction of total protein (Gene Research Lab, 2007).

Ion exchange chromatography separates proteins based on their surface ionic charge using resin that are modified with either positively-charged or negatively-charged chemical groups (Cseke et al., 2004; Watson et al., 2004). Most proteins have an overall negative or positive charge depending on their isoelectric point (pI) at a given pH, which makes them possible to interact with an opposite charged chromatographic matrix (Watson et al., 2004). If the net charge of the protein is positive at a pH below pI value, the protein will bind to a cation exchanger; at a pH above the pI value the net charge of the protein is negative and the protein will bind to an anion exchanger (Wheelwright, 1991).

Gel filtration chromatography, also called size-exclusion or gel-permeation chromatography, which employs the principle of gel filtration chromatography described by Bangalore Genei (2007) separates proteins according to molecular sizes and shapes and the molecules do not bind to the chromatography medium (Gene Research Lab, 2007). Another method, Affinity chromatography hinges on a specific interaction between the protein and the solid phase to affect separation from contaminants. It enables the purification of a protein on the basis of its biological function or individual chemical structure (Amersham Biosciences, 2002).

Gel electrophoresis separates protein according to their size and charge properties. The partially purified protein from the chromatography separations can be further purified with nondenaturing polyacrylamide gel electrophoresis (PAGE), or native gel electrophoresis (Cseke et al., 2004). In PAGE, the proteins are driven by an applied current through a gelled matrix (Karp, 2008). The movement of protein through this gel depends on the charge density (charge per unit of mass) of the molecules. The molecules with high density charge migrate rapidly. The size and shape of protein are another two important factors that influence PAGE fractionation (Karp, 2008). SDS used in electrophoresis resolve mixture of proteins according to the length of individual polypeptide chains (Watson et al., 2004). A technique, two-dimensional gel electrophoresis was developed by Patrick O'Farrell in 1975. It is used to fractionate complex mixtures of proteins by using two

different techniques-isolectric focusing and SDS-PAGE (Tan and Yiap, 2009). These methods separate proteins first according to their isoelectric point in a tubular gel and then according to their molecular mass on SDS-saturated polyacrylamide slab (Karp, 2008). Two-dimensional gel electrophoresis is suitable to detect changes in proteins present in a cell under different conditions, at different stages in development or the cell cycle, or in different organisms (Tan and Yiap, 2009). Automated extraction system has helped to simplify the isolation of nucleic acids with beneficial advantages including increasing reproducibility and quality of results (Boyd, 2002; Promega Corporation, 2008).

MICRO-COMPARTMENTS UNIQUE TO PROKARYOTES

Magnetosomes

Magnetotactic bacteria (MTB) for example, *Magnetospirillum magneticum* are several micrometer long, aerobic, flagellated, Gram negative bacteria (Xie et al., 2005). The most remarkable characteristic of MTB is that it contains intracellularly synthesized components called magnetosomes, which are usually made of magnetite (Fe_3O_4) or greigite (Fe_3S_4) (Bazylinski et al., 2004). Unlike most other intracellular inclusions in prokaryotes which are compartmentalized in a relatively thin monolayer of protein only (e.g. sulfur globules) (Shively, 1974), the magnetosome membrane is a true phospholipid bilayer (Gorby et al., 1988).

Techniques for the isolation of magnetosome particles from the magnetotactic bacterial cells are based on two different types of processes. After bacterial cells are harvested by centrifugation (8000 rpm, 15 min, and 4°C), MTB can be precipitated and boiled to lyse the cells (Yang et al., 2001). Another method employed is sonication (Alphandéry et al., 2011). The optical microscope can be used to examine the effect of disruption. Magnetosomes from the disrupted cells align nearest the magnet while the nonmagnetic fluid fraction is removed by aspiration.

Purifying magnetosomes from cells is a relatively tedious process. It is however facilitated by the use of magnetic separation techniques (Bazylinski et al., 1994; Gorby et al., 1988). Precipitation and treatment in 1% sodium-dodecyl-sulfate produces individual magnetosomes which can then be separated by a magnet (Alphandéry et al., 2011).

The pyrogen content of the purified magnetosome is then assessed. Direct impedimetric method can be used for the determination of sterility of purified magnetosomes (Ghorbanzadeh-Mashkani et al., 2013).

Carboxysomes

Carboxysomes were the first bacterial microcompartments to be discovered. They are one of the best-known

examples of protein-bounded compartments in bacteria (Yeates et al., 2008), discovered as polyhedral bodies in the cytoplasm of cyanobacteria (for example, *Prochlorococcus* sp., *Synechocystis* sp.) (Cheng et al., 2008). Carboxysomes enclose enzymes involved in carbon fixation process (Badger and Price, 2003), are made of polyhedral protein shells around 80 to 140 nanometres in thickness with mass of about 300 MDa (Yeates et al., 2008) and are found in all cyanobacteria, many chemotrophic bacteria that fix carbon dioxide for example, *Halothiobacillus neapolitanus*, some nitrifying bacteria for example, *Nitrosomonas europaea*, and thiobacilli (Yeates et al., 2008).

To isolate carboxysomes from Cyanobacteria, cells are disrupted using sonication and ballotini beads (Gupta et al., 2013). Ballotini glass beads provide the grinding action while Sonication preserves the integrity of the carboxysomes. This is usually followed by centrifugation which pellets non ruptured cells, carboxysomes, and membrane fragments and separates them into gradient via sucrose gradient centrifugation. This ensures the high yield of purified carboxysomes.

Photosynthetic compartments

Photosynthetic membranes are perhaps the most thoroughly studied of all prokaryotic compartments e.g. chromatophores, which contains the various intracytoplasmic membrane (ICM) structures that house the photosynthetic protein complexes of the purple photosynthetic bacteria Murat et al. (2010), thylakoid membrane compartments found in cyanobacteria Li et al. (2001), and the chlorosome compartments of green photosynthetic bacteria (Shively, 2006). The thylakoid membranes of cyanobacteria are the evolutionary precursors of chloroplasts. As with chromatophores these compartments are responsible for some of the central light-dependent reactions of photosynthesis.

Chlorosomes

Chlorosomes are flattened, ellipsoidal structures that are connected to the cytoplasmic membranes by a relatively thick baseplate (Shively, 2006). The chlorosome envelope is 3 to 5 nm thick and electron opaque, as seen by thin-layer transmission electron microscopy (Cohen-Bazire et al., 1964; Staehelin et al., 1980). Chlorosomes are typically isolated after disrupting cells with a French pressure-cell treatment; before being isolated by ultracentrifugation on sucrose density gradients (Oelze and Golecki, 1995).

Inclusion of 2M NaSCN (Gerola and Olson, 1986) or detergents (Feick and Fuller, 1984) during the cell disruption and isolation procedure greatly enhances the separation of cytoplasmic membranes and chlorosomes. However, differences in the isolation method have been found to affect subsequent measurements on the isolated

chlorosomes (Oelze and Golecki, 1995). For example, detergents remove lipids and most of the proteins from the chlorosome envelope and, for this reason; the use of detergents to release chlorosomes from membranes is not recommended (Vassilieva et al., 2002).

In transmission electron microscopy, isolated chlorosomes from *Chlorella tepidum* appear about 110 to 180 nm long and 40 to 60 nm in diameter (Frigaard et al., 2004). With atomic force microscopy, isolated chlorosome from *Chl. tepidum* appear a little larger, about 170 to 260 nm long, 90 to 160 nm wide, and 30 to 40 nm high (Martinez-Planells et al., 2002; Frigaard et al., 2005). This layer is thinner than the cytoplasmic membrane (8 nm), indicating it is not a lipid bilayer. However, lipids have been identified in purified chlorosomes, and the chlorosome envelope fractures in freeze-fracture electron microscopy in a manner characteristic of lipids, suggesting that the envelope is a lipid monolayer (Staehelein et al., 1980; Frigaard and Bryant, 2006). Ten proteins have been purified from *Chl. tepidum* chlorosomes and all of them have been shown to be susceptible to cleavage by proteases, suggesting they are surface exposed. Antisera to these proteins can precipitate chlorosomes, further supporting the model that these proteins are in the chlorosome envelope (Chung and Bryant, 1996; Vassilieva et al., 2002). Chlorosomes are purified by a sucrose gradient centrifugation repeatedly. Isolated chlorosomes were then dialysed in 10 mM Tris-HCl buffer. Further purification step can be carried out using a flotation sucrose gradient (Steensgaard et al., 1997).

Thylakoid membrane

Thylakoid membrane are sites of photosynthesis and respiratory electron transport in cyanobacteria e.g. *Anabaena*. Isolation method for plasma and thylakoid membranes from *Anabaena* involves growing the cells until mid-exponential phase before being harvested by centrifugation (Li et al., 2001). Sonication is then first used to break the cells.

After unbroken cells are removed via low speed centrifugation, the supernatant is then passed through high speed centrifugation to retrieve the membranes that is, both the thylakoid and plasma membrane. The total membranes are then passed through two polymer system for the separating of membranes (Norling et al., 1998). Thylakoids can then be purified using a combination of differential and gradient centrifugation (Peltier et al., 2002).

Gas vesicle

Another unique protein-bounded organelle in bacteria is the gas vesicle. Gas vesicles are cylindrical or spindle-shaped with size varying between species and are gas-

filled, protein-bound compartments that function to modulate the buoyancy of cells (Walsby, 1994). The gas vesicles are found in a number of bacteria and archaea including halophilic (*Halobacterium halobium*), methanogenic archaea, phototrophic and heterotrophic bacteria (Murat et al., 2010). Most bacteria and archaea that have been shown to form gas vesicles are found in aqueous environments and are nonmotile. Gas vesicle gene clusters have been reported to exist in *Bacillus megaterium* and some members of the Actinomycete genera (van Keulen et al., 2005).

To isolate gas vesicles, cells are allowed to grow in substrate depleted medium which results in the formation of protoplast (Archer and King, 1984). Vacuolate protoplasts are separated from unvacuolate ones by flotation and the protoplast membrane removed by Tween 20, liberating the gas vesicles. The gas vesicles are then purified by flotation after initial passage through a 0.45 µm filter to remove contaminating material. Gas vesicle membranes can be purified by isopycnic gradient centrifugation (Archer and King, 1983).

Outer membrane

Cyanobacteria represent the only group of prokaryotes capable of performing plant-like photosynthesis. Most species have a differentiated thylakoid membrane, which is the site for the light-dependent reactions of photosynthesis (Huang et al., 2004). In addition, all cyanobacteria possess a cell wall consisting of an outer membrane, a peptidoglycan layer, and the plasma membrane. The method for the isolation and purification of the outer membrane of the cyanobacteria *Synechocystis* sp. is similar to that applied to the thylakoid and plasma membrane earlier discussed. Cells are broken with glass beads, and the total membrane separated by sucrose density centrifugation (Huang et al., 2002). The pellet from the sucrose gradient is then fractionated by two-phase extraction (Norling et al., 1998). To further purify outer membranes from minor cross-contamination with plasma membrane, two more partitions in the 6.6% repartitioning system is performed. Completely pure outer membranes resulting in the final top phase (T5) is collected and washed by a centrifugation at 125,000 g for 1 hour (4°C). The purity of the outer membrane fraction was verified by immunoblot analysis using antibodies against membrane-specific marker proteins (Huang et al., 2004).

Polyhydroxybutyrate

Polyhydroxybutyrate (PHB) is an aliphatic polyester biosynthesized by several bacteria for example, *Herbaspirillum seropedicae* as a means of carbon storage and source of reducing equivalents (Jendrossek,

2009). *H. seropedicae* is a diazotrophic β -Proteobacterium found associated with important agricultural crops (Tirapelle et al., 2013). This bacterium produces polyhydroxybutyrate (PHB), aliphatic polyester, as a carbon storage and/or source of reducing equivalents (Catalan et al., 2007). The PHB polymer is stored as intracellular insoluble granules coated mainly with proteins, some of which are directly involved in PHB synthesis, degradation and granule biogenesis. Tirapelle et al. (2013) extracted the PHB granules from *H. seropedicae* and identified their associated-proteins by mass spectrometry. *H. seropedicae* cells were harvested by centrifugation and the cell pellet washed once with potassium phosphate buffer before being re-suspended and sonicated in the same buffer (Potter et al., 2004). The PHB granules within the insoluble fraction were then purified by ultracentrifugation in two glycerol gradients (Tirapelle et al., 2013).

COMPARTMENTS UNIQUE TO EUKARYOTIC CELLS

Apicoplasts

The isolation of apicoplasts and detailed analysis of their lipids in relation to those of whole parasites is an essential prerequisite for understanding the steps involved in apicoplast biogenesis and identifying lipids that are potentially important for apicoplast biosynthetic functions (Botte et al., 2013). Previous attempts to isolate apicoplasts from *P. falciparum* or *Toxoplasma gondii* using density gradient centrifugation (Kobayashi et al. (2007) or capillary zone electrophoresis (Moe et al., 2010) have resulted in low yields and/or poorly defined fractions.

Botte et al. (2013) described a method to isolate and purify apicoplasts from *P. falciparum* using immunolysis, a method engaged to purify subcellular compartments from other eukaryotes. Host erythrocytes were permeabilized by saponin to release free parasites, which were then lysed by osmotic shock (Mullin et al., 2006). Nuclei and cellular debris were removed by low-speed centrifugation to generate an organelle fraction from which apicoplasts were retrieved using magnetic beads coated with an anti-HA monoclonal antibody. Apicoplast purity was assessed by Western blotting (Botte et al., 2013).

Peroxisomes and glycosomes

Peroxisomes are ubiquitous subcellular compartments of eukaryotic cells. The isolation of peroxisomes from the yeast *S. cerevisiae*, however, has been hampered by the fact that, under standard growth conditions, peroxisomes are present in low numbers. Also, the liability of peroxisomes in general has complicated their purification.

Two observations have greatly facilitated the isolation of peroxisomes from yeast: first, peroxisomes are induced by growth on a fatty acid (Veenhuis et al., 1987), and second, peroxisomes are more stable at low pH (~5.5). The method employed involves osmotic lysis of yeast spheroplasts at low pH. Digestion of the yeast cell wall with zymolyase is optimal at pH 7.5 and since yeast peroxisomes are unstable at this pH and must be isolated at pH 5.5 to 6.0, the use of zymolyases is unsuitable for cell disruption. If lysis is not complete, spheroplasts can be sheared gently using a Dounce homogenizer.

Following cell lysis, differential centrifugation to obtain an organelle pellet is done (Goodman, 1985). Pellet obtained is layered on a discontinuous sucrose gradient to separate mitochondria and peroxisomes because of their relatively high equilibrium density in sucrose (~1.24 g/cm³). As with all compartments, peroxisomes can be purified from cell lysates using a combination of differential centrifugation and density gradient centrifugation. Peroxisomes purified in this way are relatively stable and show only minor contamination with mitochondria. Membrane-bound cytoplasmic structures that resemble those initially designated as microbodies and later on as peroxisomes in mammalian cells, have been described in trypanosomatids since the initial studies on their fine structure (De Souza, 1984). The peroxisomes usually appear as spherical compartments with a diameter of about 0.7 μ m and are randomly distributed throughout the cell.

In some cells, as in *L. samueli*, they appear as elongated structures that can reach a length of 2.8 μ m (Souto-Padrón and De Souza 1982). Opperdoes et al. (1984) obtained a highly purified subcellular fraction containing glycosomes from bloodstream-form trypanosomes of *T. brucei*: grinding with silicon carbide disrupted the parasites and a glycosome enriched fraction was recovered from a Percoll gradient. The organelle was further purified on a sucrose gradient, equilibrating at 1.23 g/cm³ (Opperdoes et al., 1984). Purified glycosome fractions were permeabilized with toluene (McLaughlin 1985) or Triton X-100 after treatment with cross-linking agents (Aman et al., 1985), to test the activity of core enzymes.

Golgi complex

The golgi apparatus of the eukaryotic cell is crucially involved as the main secretory protein processing factory in intracellular vesicular transport (Inadome et al., 2005). It plays a central role in the endomembrane system of eukaryotes. The Golgi complex of trypanosomatids is formed by 4 to 10 stacked cisternae localized in the anterior region of the cell, close to the flagellar pocket (de Souza and Cunha e-Silva, 2003). Two attempts have been made to purify the Golgi complex of trypanosomatids. The parasites were suspended in a hypotonic solu-

tion containing protease inhibitors and then disrupted by controlled sonication followed by centrifugation at 95 000 × *g* for 90 min in a discontinuous sucrose density gradient (1.2, 1 and 0.8 M sucrose) (Morgado-Díaz et al., 2001). Transmission electron microscopy showed that the fraction consisted predominantly of smooth surface vesicles and flattened cisternae rather than stacked Golgi cisternae. A band recovered at a position corresponding to the 1 to 1.2 M sucrose interfaces was highly enriched in stacked cisternae and vesicles. The fraction was characterized biochemically as significantly enriched in galactosyl transferase, O- α -Glc NAc transferase and acid phosphatase (Morgado-Díaz et al., 2001).

Acidocalcisome

Acidocalcisomes are membrane-bounded compartments with an electron-dense content, acidic character and calcium storage capacity (Docampo and Moreno, 1999). Scott et al. (1997) made the first attempt to isolate acidocalcisomes from *T. cruzi* epimastigotes. Treatment with Triton WR-1339 was done to decrease the density of intracellular vacuoles, before lysis using grinding with silicon carbide.

The compartments were then separated on a Percoll gradient. An improvement was made by Scott and Docampo (1998) omitting the detergent treatment. Similar methods have been adopted to isolate acidocalcisomes from *L. donovani* (Rodrigues et al., 1999a) and *T. brucei* (Rodrigues et al., 1999b). A new procedure was established by Scott and Docampo (2000). It was an improvement on earlier methods which had resulted in great loss of acidocalcisomes when the percoll gradient was washed. The improved protocol involved using iodixanol, a density gradient solute that does not precipitate, which allowed acidocalcisome isolation in good yield.

Proteasomes

The proteasome is a 2.5 megadalton protease, present in all eukaryotes that perform the bulk of non-lysosomal degradation of aberrant, damaged, misfolded, and naturally short lived regulatory proteins in eukaryotic cells as well as proteins conjugated to ubiquitin (Masters et al., 2005). Various methods are employed for extraction of proteasomes from yeast cells. They are the affinity-based purification which is much quicker than the conventional method, requiring only 4 h instead of several days that Conventional purification protocols involve. The cells are harvested by centrifugation and lysed by passing the pellets through French press for yeast cells or French pressure cell (two passes, minimum 5000 psi) followed by three rounds of 30 s of sonication for *E. coli* cells. The lysate is then clarified through ultracentrifugation. The lysate is filtered through cheesecloth to remove any lipids that float on the surface of the lysate after centrifugation

and the supernatant is 0.2 μ m filtered. After washing with TBS, protein is eluted and fractions is monitored using SDS-PAGE and is then pooled and concentrated by ultrafiltration. Purified proteasome is assessed for purity and distribution by native gel electrophoresis (Patterson and Cyl, 2005).

Vacuoles

In yeast, the vacuole corresponds to the mammalian lysosome. In contrast to the rather small mammalian lysosome, yeast vacuoles are large (>500 nm in diameter) and present in one to five copies per cell (Cabrera and Ungermann, 2008). The purification procedure consists of an initial incubation step of the cells in 10 mM DTT at pH 9.4 to break disulfide bonds in the cell wall, followed by the digestion of the cell wall with the help of the enzyme lyticase, and the DEAE-dextran mediated gentle lysis of the cells (Bankaitis et al., 1986; Haas, 1995). Purified vacuoles are obtained by flotation in a Ficoll step gradient (Cabrera and Ungermann, 2008). Sarry et al. (2007) described another method for vacuole extraction and purification.

The cell wall is digested away from the yeast spheroplast using the enzyme zymolyase, isolated spheroplasts were centrifuged again at 3 000 rpm at 4°C for min. Density gradient purification is used to obtain high-purity “proteomics-grade” intact vacuoles. It involves loading isolated spheroplasts onto six ice-cold gradient tubes which consists of three layers: sucrose/Ficoll Buffer filled the bottom layer, prepared with sucrose Buffer with 2.5% Ficoll (w/v). Vacuoles were collected at the sucrose/Ficoll and 2:1 sorbitol: sucrose interface, washed in sorbitol buffer, and centrifuged at 3,000 rpm for 20 min.

Endoplasmic reticulum

All eukaryotic cells have an endoplasmic reticulum (ER). Its membrane typically constitutes more than half of the total membrane of an average animal cell (Alberts et al., 2002). Regions of ER that lack bound ribosomes are called smooth endoplasmic reticulum, or smooth ER. In the great majority of cells, such regions are scanty and are often partly smooth and partly rough. To study the functions and biochemistry of the ER, it is necessary to isolate the ER membrane although the ER is intricately interleaved with other components of the cytosol. However, when cells are disrupted by homogenization, the ER breaks into fragments and reseals into many small (~100 to 200 nm in diameter) closed vesicles called microsomes, which are relatively easy to purify. Microsomes derived from rough ER are studded with ribosomes and are called rough microsomes. The ribosomes attached to rough microsomes make them denser than smooth microsomes hence, the rough and smooth microsomes can be separated from each other

by equilibrium centrifugation (Alberts et al., 2002).

To isolate ER from Yeast cells, they are firstly harvested by low-speed centrifugation. The mycelia is then collected by filtration with or without protease inhibitors and lysed with glass beads (0.45 diameter) in an MSK cell homogenizer. Homogenates are centrifuged and the resulting supernatant was further centrifuged at high speed. The high-speed supernatant is collected and further separated by ion exchange chromatography on a DEAE column. The sample was eluted with a discontinuous gradient with a salt-containing buffer (Mora-Montes et al., 2010).

CONCLUSION

Isolation and purification of compartments and intra protoplasmic contents is essential for understanding their activity hence it is important to employ methods that will ensure the intactness of the compartments. Such cell disruption methods vary from organisms to organisms depending on the toughness of their cell wall or protoplasm. Methods include ballotini beads, sonication, enzyme lysis etc. most compartments are easily purified directly from isolation while others require several process of centrifugation to get them in pure form.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Short Communication

A note on interaction and pre-implantation development stages

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Received 10 June, 2014; Accepted 25 June, 2014

Having obtained observations of gene expression profiles from the literature, we estimated autoregressive models of mouse preimplantation development and observed interaction among stages. Gene expression profile at the earliest stage is not generally significant, but for observations where profiles at 8-cell embryo and morula stages are relatively great, the effect of unfertilized egg is significantly positive.

Key words: Cellular biology, embryology, gene expression, interaction, linear regression, multivariate analysis, preimplantation development, temporal changes.

INTRODUCTION

Preimplantation development is important to reproductive biology applications including livestock breeding and treatments that repair defective tissues (Hamatani et al., 2004; Wang and Dey, 2006; Chakrabarty et al., 2007), and developmental biology may be studied with genomics (Ko, 2001), Canestro et al. (2007). More specifically we can learn from changes in gene expression during preimplantation development in the mouse (Tanaka et al., 2000; Zeng and Schultz, 2003; Wobus and Boheler 2005).

This research note describes statistical analysis and results of global gene expression profiles at preimplantation development stages. Regression models of stage profiles were estimated as linear functions of earlier stage profiles, and investigation of insignificant variables led us to discover significant interaction between preimplan-

tation development stages. This interaction is important to understanding temporal changes during preimplantation development.

METHODOLOGY

We obtained 21,940 observations of gene expression profiles across the following stages of preimplantation development and assigned to them variables names (in parentheses): unfertilized egg (a), fertilized egg (b), 2-cell embryo (c), 4-cell embryo (d), 8-cell embryo (e), morula (f), and blastocyst (g). The raw data are Supplemental Data for Hamatani et al. (2004): "Embryos were collected from super-ovulated C57BL/6J mice by the standard method. MRNAs were extracted using a Quickprep micro

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Table 1. Independent variable coefficient values versus models of preimplantation stages.

Response	b	c	d	e	f	g
Constant	0.184	0.219	0.0497	0.314	-0.054	0.027
Coefficient a	0.941	-0.199	0.134	0.931	-0.140	0.009
Coefficient b		1.130	-0.0214	-0.887	0.121	0.129
Coefficient c			0.874	0.287	0.080	-0.151
Coefficient d				0.568	0.384	-0.069
Coefficient e					0.574	0.460
Coefficient f						0.630

poly-A RNA Extraction Kit and linear acrylamide as a carrier. Intensity was extracted from scanned microarray images using Feature Extraction 5.1.1 software (p. 130).” For our research, main effects models of each stage were estimated as functions of the previous stages. Where insignificant variables were found, we fit the full second-order model to investigate interaction between main effects.

RESULTS

Preimplantation development stages up to and including the morula enjoyed consistently significant relationships with previous stages in the main effects models of global gene expression profiles. Table 1 shows coefficient values of the first five linear regression models that did not require investigation into interaction (see Responses b through f), and the final model of blastocyst which is not a significant function of the gene expression profile at the unfertilized egg stage (see Coefficient a).

Based on the fact that we cannot reject the hypothesis that co-efficient a = zero in a model of the blastocyst stage, we feel obliged to consider interaction with the full second-order model. While the unfertilized egg stage may not be generally significant to the blastocyst stage, significance may be found for relatively great and/or small gene expression profiles at other stages. In the full second-order model of blastocyst stage the unfertilized eggs stage interacts significantly with 8-cell embryo (e), and morula (f). To further investigate interaction, we used k-means clustering, separated observations according to gene expression profiles at the 8-cell embryo and morula stages, and refit models of the blastocyst stage separately for each cluster.

For observations, where gene expression profiles at 8-cell embryo and morula stages are relatively great the effect of unfertilized egg was positively significantly. For observations where gene expression profiles at 8-cell embryo and morula stages are relatively small, the effect of unfertilized egg was negatively significant. In order to make the distinction between relatively great and small gene expression profiles at 8-cell embryo (e) and morula

(f) stages, we fit a linear probability model of the binary cluster variable as a function of e and f:

$$E(\text{cluster}) = -1.547 + 0.378e + 0.274f \quad (1)$$

Figure 1 of fitted values versus observations of the cluster confirms that a cut-point of 0.5 is reasonable. In other words, whenever the fitted value associated with a new observation and Equation 1 is greater than 0.5 one should assume the effect of unfertilized egg on blastocyst is positively significant, and an appropriate model for prediction of temporal changes. When the fitted value associated with a new observation and Equation 1 is less than 0.5 it is assumed that the effect of unfertilized egg on blastocyst is negatively significant, and an appropriate model.

We refit separate models of blastocyst according to the 0.5 rule described above and verified the interaction. Table 2 shows significant independent variable coefficient values for the separate models. They are consistent but for the opposite signs associated with unfertilized egg.

DISCUSSION

Articles which have used statistical analysis to study preimplantation development include studies on the early stages of the mouse embryo (Fleming and Pickering, 1985). Jurisicova et al. (1996) investigated the association between expression in human embryos and factors known to influence *in vitro* fertilization pregnancy outcomes. Takai et al. (2000) explained how prenatal exposure to an estrogenic compound alters postnatal development. Cervero et al. (2004) studied body weight and reproductive function during human embryonic preimplantation development.

We found a textbook case of interaction among preimplantation development phases. Gene expression profile at the earliest stage is not generally significant in a main effects model of the final stage. However, for observations where gene expression profiles at 8-cell embryo and morula stages are relatively great (small), the effect of unfertilized egg is positive (negative)

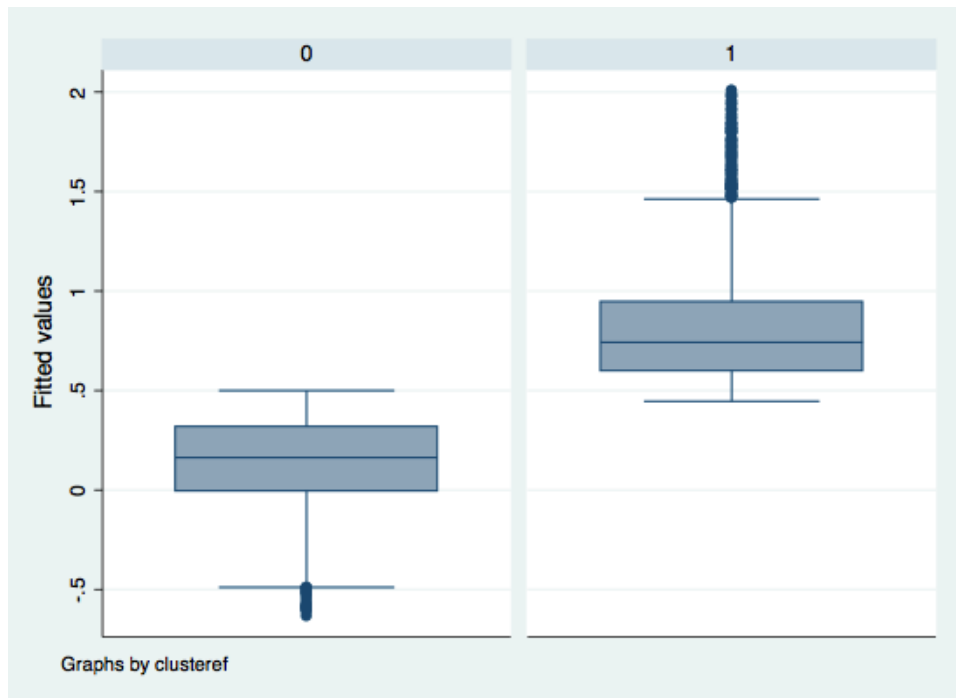


Figure 1. Fitted values versus observations of cluster.

Table 2. Independent variable coefficient values versus models of the blastocyst stage.

Response	g (relatively small e and f)	g (relatively great e and f)
Constant	0.107	-0.078
Coefficient a	-0.019	0.050
Coefficient b	0.127	0.024
Coefficient c	-0.127	-1.645
Coefficient d	-0.027	-0.115
Coefficient e	0.385	0.493
Coefficient f	0.622	0.674

significant. Future work should justify assumptions of the statistical models or search for more theoretically appropriate ones. Also, we hope other authors like Hamatani et al. (2004) will publish their data making it possible to verify these results and discover new ones.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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