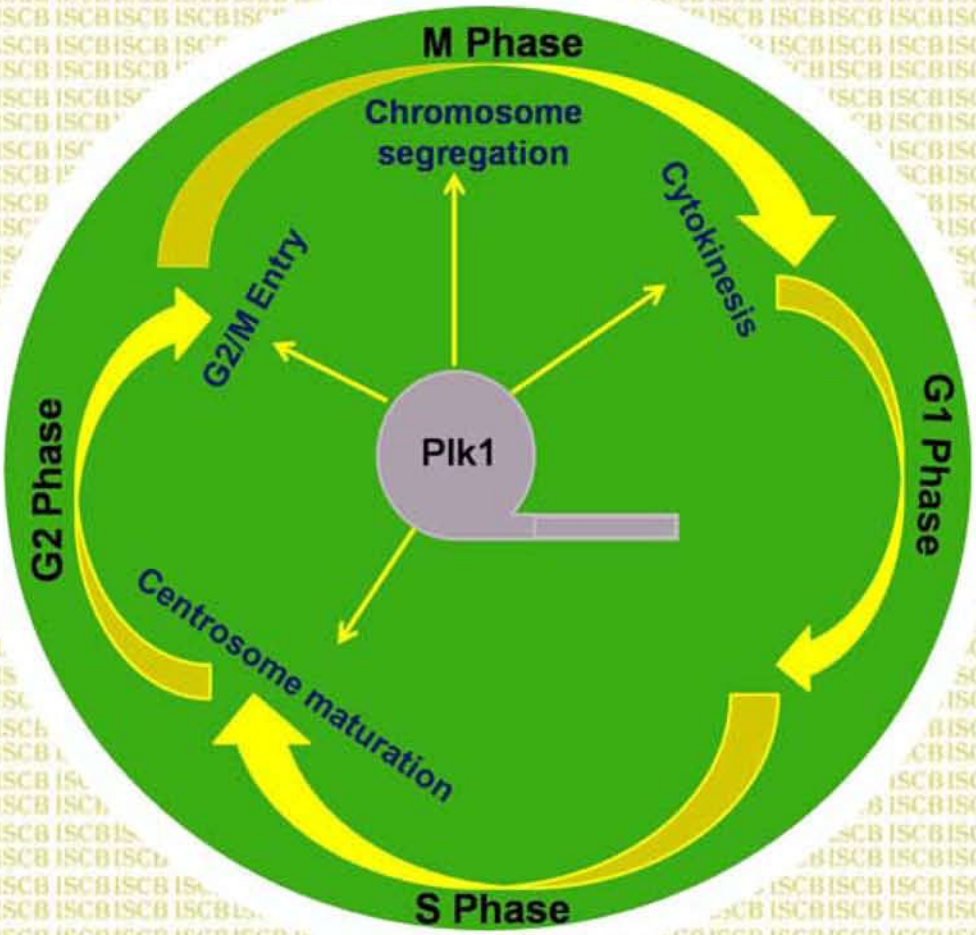
Volume 33:2 ISSN 2349-8307 September 2014

CELL BIOLOGY NEWSLETTER



Published by
INDIAN SOCIETY OF CELL BIOLOGY
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Cover: Polo game is divided into three to six chukkas and the gaps in between them is generally used to change ponies etc. The cell cycle depicted in green colour resembles a polo field where the central player of the game, Plk1 performs its various functions such as centrosome maturation, G2/M entry, chromosome segregation and cytokinesis in various chukkas at different time points of the cell cycle.

INDIAN SOCIETY OF CELL BIOLOGY (Regd.)

Prof B N Singh
President
Genetics Laboratory
Department of Zoology
Banaras Hindu University
Varanasi 221 005

Telephone : (0542)2368145 (Lab), 09935345196 (Mobile); FAX : (0542)2368457; E-mail : bnsingh@bhu.ac.in

26th August 2014

Dear Members,

I am happy to let you know that the Newsletter of the Indian Society of Cell Biology has been assigned an International Standard Serial Number (ISSN-23498307). This is a matter of great pleasure for all of us. From this, the Newsletter of the Society attains international identity which will enable the researchers to refer the journal. This will also enhance the visibility of the Society as well as its Newsletter at the national and international levels.

I request you all to contribute your timely, interesting and relevant articles in the area of Cell Biology to the Newsletter of the Society.

With best wishes,

B N Singh

From the Secretary's Desk

Dear Members of the ISCB,

We have great pleasure in bringing out the next issue of the ISCB Newsletter for the year 2014. This is the first issue that has an ISSN number. The ISSN number gives unique international identity to our publication and enables easy referencing. This volume has four interesting articles on topics of interest to cell biologists. Ms. M. Babuta has concisely put together findings on the role of polo like kinase in cell division. Ms. Rangaraj, through her article, updates us on the latest imaging techniques available and how they have helped us have detailed knowledge of molecular function within cells. Dr. Ramanan gives us an update on how protoplasts can be used to understand cellular processes in rice plants. Prof. B.N. Singh has provided us with insights into the complex chromosomal polymorphism seen in *Drosophila ananassae*. The issue also has a write-up of the proceedings of the APOCB meeting held at Singapore in February 2014. We thank all the contributors for submitting articles to the Newsletter.

We are happy to announce that Dr. Satyajit Mayor, Director NCBS has been elected as President of APOCB for the term 2014-2018 and the next meeting of APOCB will be held in India in January 2018. The APOCB, the ASCB and IFCB have extended support to enable members of ISCB to attend their international meetings and engage with cell biologists from across the world. This year, the Annual Conference of our Society will be held at CDRI Lucknow from 10th to 12th December. The S.P.Ray-Choudhary 75th Birthday Endowment Lecture will be delivered during this meeting.

The term of the present Executive Committee will end in March 2015. The process for election of new office bearers is on. We thank Dr. Veena Parnaik for consenting to take up the duties of the Returning Officer. Request your active participation in the electoral process. For the first time we are conducting the electoral process totally through electronic mail. One of the reasons for doing this is to enhance participation of members in the election process (which we found was really dwindling when members were required to physically post nominations and the ballot).

Exposure of students to concepts in cell biology has been one of the activities of our Society. Towards this end, several members of the Society from Hyderabad, conducted a one-day workshop for biology teachers and students from 20 schools in and around Hyderabad. This activity was carried out as part of the Golden Jubilee celebrations of St. Ann's High School, Vijayanagar Colony, Hyderabad. We encourage members of the society to engage in similar activities.

This issue of the Newsletter also has the audited Statement of Accounts of the Society for the financial year 2013-2014.

Vegesna Radha
Secretary

A.J. Rachel
Joint Secretary

Features of the pattern of the chromosomal polymorphism in *Drosophila ananassae*

B N Singh
Genetics Laboratory
Department of Zoology
Banaras Hindu University
Varanasi-221005
E-mail:bashisthsingh2004@rediffmail.com, bnsingh@bhu.ac.in, bnsinghbhu@gmail.com

In the genus *Drosophila*, chromosomal polymorphism due to paracentric inversions is very common and is an adaptive trait (see Singh 1994, 1996,1998a,b, 2013). However, pericentric inversions and translocations are also known in many species of *Drosophila* (Singh 1994). *Drosophila ananassae* is a cosmopolitan and domestic species. It is characterized by several unusual genetic features (Singh 1985a, 1996, 2000, 2010). Its common occurrence in India coupled with its genetic peculiarities attracted the attention of Indian researchers. Genetical studies in *D. ananassae* in India were initiated by Prof S. P. Ray-Chaudhuri (First President of the Indian Society of Cell Biology:1977-78) in 1940s at the Department of Zoology, Calcutta University. When he moved to the Department of Zoology, Banaras Hindu University in 1960, Prof Ray-Chaudhuri continued his research on *D. ananassae* with particular reference to population genetics and male crossing-over. Polytene chromosome maps of *D. ananassae* were constructed and a number of chromosomal aberrations were detected (Ray-Chaudhuri and Jha 1966). Spontaneous male crossing-over in *D. ananassae* was found to be meiotic in origin (Ray-Chaudhuri and Kale 1966; Kale 1969). It was also observed that chromosomal polymorphism in *D. ananassae* is balanced due to adaptive superiority of inversion heterozygotes (Singh and Ray-Chaudhuri 1972). Population genetical studies with particular reference to population dynamics of inversion polymorphism and related phenomena are still being pursued at the Department of Zoology, Banaras Hindu University. In this note, the main features of the pattern of chromosomal polymorphism in *D. ananassae* are briefly described:

1. It exhibits a high degree of chromosomal polymorphism in its natural populations (Singh 1988a, 1998a,b; Singh and Singh 2007a). In total there are 78 paracentric inversions, 21 pericentric inversions and 48 translocations. The occurrence of pericentric inversions and translocations is rare in other species of *Drosophila* and reflects unusual mutational properties of *D. anana*ssae. It has been suggested that it has developed some special mechanisms through which it can retain in its natural populations the chromosome arrangements which are disadvantageous. A new inversion within the subterminal inversion in 2L of *D. ananassae* was detected in a laboratory stock originating from Kuala Lumpur, Malaysia and testing of Hardy-Weinberg Equilibrium showed the presence of heterosis associated with inversion heterozygotes (Singh 1983).

2. Although a large number of paracentric inversions are known to occur in this species, only three have become coextensive with the species and have been called as cosmopolitan inversions by Futch (1966). Most of the inversions have localized distribution and have been detected from a few individuals. This is a feature of the pattern of the chromosomal polymorphism in *D. ananassae* (Carson 1965). The cosmopolitan distributions of the three inversions (AL in 2L, DE in 3L and ET in 3R) have been considered from the view point of monophyletic origin of these inversions (Singh 1970).

3. The results of population cage experiments have shown that chromosomal polymorphism in *D. ananassae* is balanced due to adaptive superiority of inversion heterozygotes (Singh and Ray-Chaudhuri 1972). The cosmopolitan inversions often persist in laboratory stocks which provides evidence that heterotic buffering is associated with the three cosmopolitan inversions (Singh 1982). Although inversion polymorphism is subject to selection, inversion frequency may change in laboratory populations due to random genetic drift (Singh 1987, 1988b; Singh and Singh 2008a).

4. There is persistence of heterosis associated with these three inversions in interracial hybridization experiments which has been explained by suggesting that there is absence of genetic coadaptation in geographic populations of *D. ananassae* (Singh 1972, 1985b). The heterosis associated with inversions in *D. ananassae* appears to be simple luxuriance rather than populational heterosis (coadaptation).

5. Extensive data on the frequencies of inversions in Indian natural populations of *D. ananassae* have been reported (Singh 1984, 1989, 1996, 1998b; Singh and Singh 2007b, 2008b). Based on the values of genetic identity (I) and distance (D), it has been suggested that natural population of *D. ananassae* have undergone considerable degree of genetic divergence at the level of chromosomal polymorphism as a consequence of their adaptation to varying environments and natural selection operates to maintain the three cosmopolitan inversions. In general, south Indian populations including Andaman and Nicobar Islands show high frequency of inversions.

6. Population structure analysis in forty five Indian natural populations of *D. ananassae* was performed by employing three cosmopolitan inversions as markers for the first time (Singh and Singh 2010). Pairwise F_{ST} analysis and genetic distance (D) values showed strong genetic differentiation among populations. Values of gene flow based on F_{ST} estimates are very low ($Nm<5$). The strong genetic differentiation and minimal gene flow indicate strong sub-structuring in Indian natural populations of *D. ananassae* at the level of inversion polymorphism.

7. Singh and Chatterjee (1986, 1988) studied the mating ability of homo- and heterokaryotypes due to subterminal (AL-2L) inversion derived from natural populations of *D. ananassae* in which frequency of different chromosome arrangements were known. Their main conclusions are: (i) the chromosome occurring in high frequency is associated with higher mating activity in all the populations analyzed; (ii) the heterokaryotypic males are superior in mating propensity to the corresponding homokaryotypes, indicating the existence of heterosis associated with the AL inversion with respect to male mating activity; and (iii) males show greater variation than females which indicates striking sex difference in *D. ananassae*. Thus, inversion polymorphism may have partial behavioural basis in *D. ananassae*.

8. There is evidence for incipient sexual isolation between karyotypically different homozygous strains of *D. ananassae* derived from natural populations which shows that chromosome arrangements may correlate with mate recognition system in *D. ananassae*. This is the first report in which behavioral isolation has been found between two karyotypically different homozygous strains of *D. ananassae* derived from the same geographic location. The results of this study also extend evidence for instability of mate recognition system within *D. ananassae* (Nanda and Singh 2011).

9. There is evidence for one-sided rare male mating advantage (frequency dependent sexual selection) associated with AL/AL males in *D. ananassae* (Som and Singh 2004).

10. Yadav and Singh (2003, 2006) tested the effect of chromosome arrangement frequencies on body size in *D. ananassae*. Results have shown that polymorphic inversions and their combinations affect body size (thorax length) differently in different selection lines. Thus thorax length in *D. ananassae* is under polygenic control and inversion polymorphism plays crucial role in maintaining body size by modifying genotypic frequencies under various selection pressures (Yadav and Singh 2006).

11. There are intra- and interchromosomal effects of chromosome inversions on crossing-over in *D. ananassae* (Singh 1973; Singh and Singh 1987, 1988a,b; Singh and Mohanty 1990, 1991). Crossing over also varies in different strains. Inversion heterozygosity in one chromosome enhances crossing-over in the other chromosome. When crossing over was studied cytologically, two linked inversions of second chromosome (AL-ZE) and of third chromosome (DE-ET) strongly suppress crossing over between them when heterozygous in spite of long chromosome distance available for crossing over between them. Thus there is no correlation between chromosome distance and crossing over between heterozygous inversions in *D. ananassae* when studied cytologically. The mean heterozygous inversions is very low and suppression of crossing over between heterozygous inversions is more in *D. ananassae*. Thus it has been suggested that strong suppression of crossing over between inversions is the genetic characteristic of *D. ananassae* and may be advantageous for a species with a considerably low level of inversion heterozygosity in its natural populations (Singh and Mohanty 1990).

12. Chromosome inversions provide a mechanism for maintaining heterotic systems through the suppression of crossing over. Particularly interesting in this regard is the occurrence of non-random associations (linkage disequilibrium) of linked inversions in many species of *Drosophila*. Although linkage disequilibrium between inversions was reported for the first time by Levitan (1958) in *D. robusta*, this phenomenon which is of considerable evolutionary significance has been reported in many species of *Drosophila*. Factors causing linkage disequilibrium between inversions vary in different species and also in different chromosomes of the same species. The factors which generate linkage disequilibrium are selection, genetic drift, tight linkage, founder effect, migration, gene flow and genetic hitchhiking (Singh 2008). In a study conducted by Singh and Singh (1990), four natural populations of *D. ananassae* were sampled and data on the combinations of 3L and 3R karyotypes were obtained. For all the four populations, laboratory stocks (mass cultures and isofemale lines) were established and maintained in the laboratory. After ten generations, chromosomes of all the stocks were sampled. It was found that the two linked inversions (DE-ET) of the third chromosome are associated randomly in natural populations and mass culture stocks. On the other hand, the same two inversions show non- random association (linkage disequilibrium) in several isofemale lines. This result suggests that random genetic drift is the cause of linkage disequilibrium in isofemale lines. The tight linkage between the two inversions as evidenced by recombination studies, support the notion that linkage disequilibrium is caused by random genetic drift (Singh and Singh 1990).

Based on the above findings, it is concluded that *D. ananassae* is characterized by a number of interesting features of the pattern of chromosomal polymorphism. By employing chromosome inversions, a number of phenomena which are of considerable evolutionary significance have been elucidated in this genetically unique species.

Acknowledgements

I thank the University Grants Commission, New Delhi for the UGC-BSR Faculty Fellowship Award and the Banaras Hindu University for the appointment of Professor Emeritus.

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Polo like kinase1: A multifaceted kinase involved in cell division

Mrigya Babuta
School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067

Introduction

In eukaryotic cells, equal distribution of duplicated genome among their daughter cells involve error free multiple steps. These steps includes Interphase (G1, S and G2) the phase of genome duplication and preparation of cell to enter the Mitotic phase (M-phase). In M phase, kinetochores of sister chromatids attached to bipolar spindle and chromosomes are segregated. M phase is followed by cytokinesis which is marked by the formation of spindle midzone and cleavage furrow ingression which leads to the formation of two daughter cells.

Master regulators of cell division known from long time are cyclins and cyclin dependent kinases (cdks). Intriguingly, the progression of cell division not only depends on the fine tuning of different cyclin and their cdks but also on many others recently discovered protein kinases such as Aurora kinase and polo like kinase.

Polo like kinase was first identified in *Drosophila melanogaster* where the POLO mutants showed defects in spindle formation[1] and played a crucial role in progression of mitosis[2]. Later it was revealed that polo gene encoded a kinase[2] which was highly conserved from yeast to mammals. In yeast there is single copy of Polo like kinase namely cell division cycle 5 (Cdc5)[3] in *Saccharomyces cerevisiae* (budding yeast) and Plo1[4] in *Schizosaccharomyces pombe* (fission yeast). In *Xenopus laevis* three member of Polo like kinase family exists namely Plx 1, Plx2 and Plx 3, where Plx1 is required at multiple points during mitotis[5, 6]. In mammals four members exists i.e. Plk1, Plk2 (or SNK), Plk3 (or Fnk/Prk) and Plk4 (or Sak) [7] as shown in Fig 1(a). Plk1 performs most of the function of polo like kinase of other organisms such as Cdc5, Plo1 and Plx1, so this review focuses on the role of Plk1 during different stages of cell cycle.

A)

Name of Organism	Polo like kinase family members
Saccharomyces cerevisiae (budding yeast)	Cdc5
Schizosaccharomyces pombe (fission yeast)	Plo1
Xenopus laevis	*Plx 1* *Plx2* *Plx 3*
Mammals	*Plk1,* *Plk2 (or SNK),* *Plk3 (or Fnk/Prk)* *Plk4 (or Sak)*

B)

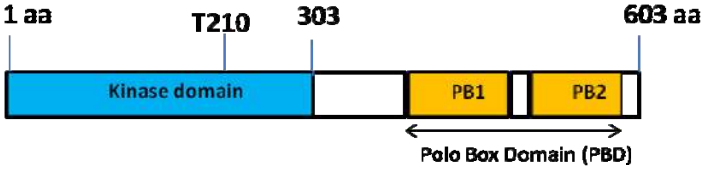

Fig 1. Polo like kinase family members and domain organization. (A) Table representing the polo like kinase family members in different organisms. B) Schematic representation of domain organization of Plk1 comprising of kinase domain and Polo box domain

Polo like kinase is a serine/threonine kinase with N-terminal kinase domain which is highly conserved and C-terminal Polo box domain (PBD) as shown in Fig 1(b). Polo box domain comprises

of two polo box regions in mammalian Plk1, Plk2 and Plk3, however the Plk4 has only single polo box region[8].

Plk1 like other kinases are regulated primarily by phosphorylation and by its polo box domain. Polo like kinases in its inactive form attains a conformation where PBD binds to the T-loop region (which is present at the amino terminal in kinase domain) and makes it unavailable for phosphorylation[9]. Phosphorylation at threonine 210 by the upstream kinase causes the activation of Plk1[9]. PBD structure[10] and proteomic screen[11] identified the role of PBD as phospho peptide binding motifs where they recognized Ser-[pSer/pThr]-[Pro/X] motif and helped in targeting Plk's to different subcellular localization and to different mitotic substrates.

Plk1 mainly start expressing from G_2 phase and remains up to cytokinesis[12]. The detailed functions of Plk1 at different stages are as follows:

Role of Plk1 in Centrosomes

Centrosomes are cytoplasmic organelles which comprises of a pair of barrel like structure known as centrioles. Centrosomes are the main hub of microtubule organization where microtubules attach and are nucleated. Centrioles are surrounded by the pericentriolar matrix (PCM) and during the course of cell cycle there is an expansion of PCM which is known as centrosome maturation. Centrosome maturation is essential process for the cell preparing for mitosis because during this process essential cell cycle regulating proteins, microtubule nucleating proteins and several other proteins enriches at the centrosomes [13, 14, 15].

Role of Polo like kinase in spindle pole formation is known from their time of discovery where polo mutant in Drosophila had defect in mitotic spindle formation with broad poles[1]. However the question remained unanswered was how the Plk1 regulates centrosome maturation.

Recent advances have shown that Plk1 is involved in microtubule assembly by regulating centrosomal protein Nlp i.e. ninein-like protein. Nlp interacts with γ- tubulin and γ- tubulin ring complex (γ-TURC comprises of three proteins namely GCP4, GCP5 and GCP6) which enriches at the centrosomes during its maturation[16, 17]. Plk1 phosphorylates Nlp which leads to the dissociation of Nlp from centrosomes and γ-TURC thus increasing microtubule nucleation drastically[18].

Other reports also established the role of Plk1 in centrosome maturation and in maintaining spindle pole integrity. Plk1 phosphorylates a PCM protein i.e. pericentrin (PCNT) at Ser1235 and Ser1241 which helps in the recruitment of PCM proteins at the centrosomes leading to its maturation at the onset of M phase[19]. Also Plk1 regulates a centrosomal protein kizuna whose depletion causes fragmentation and dissociation of PCM. Plk1 phospharylates kizuna at threonine 379 (Thr 379) residue, provides stability and helps in maintaining the architecture of centrosome [20]. Plk1 also controls the localization of other proteins such as Aurora A and TPX2 at the centrosome which are involved in centrosome maturation and microtubule nucleation respectively [21]. Therefore Plk1 plays an indispensible role in centrosome maturation, microtubule nucleation and maintaining spindle pole stability.

Role of Plk1 in Mitotic Entry and G2 DNA-damage induced arrest

Cell cycle transition from one phase to another depends on the specific set of cyclin and cyclin dependent kinase (CDK). Entry into M-phase requires Cyclin B and cdk1 complex, where cyclin B is accumulated at G2 phase[22]. However, cyclin B-cdk1 complex remains in inactive state due to the phosphorylations at Threonine (Thr 14) and Tyrosine 15 (Tyr 15) residues of cdk1 by Myt1 and Wee1 respectively[23]. Activation of this complex occurs when dual specificity Cdc25 phosphatase removes the inhibitory phosphorylation from Cdk1[24]. Once cyclin B-cdk1 complex is active via positive feedback loop it further phosphorylates Wee1/Myt1 and cdc25 which in turn inhibits and activate them respectively as shown in Fig 2.

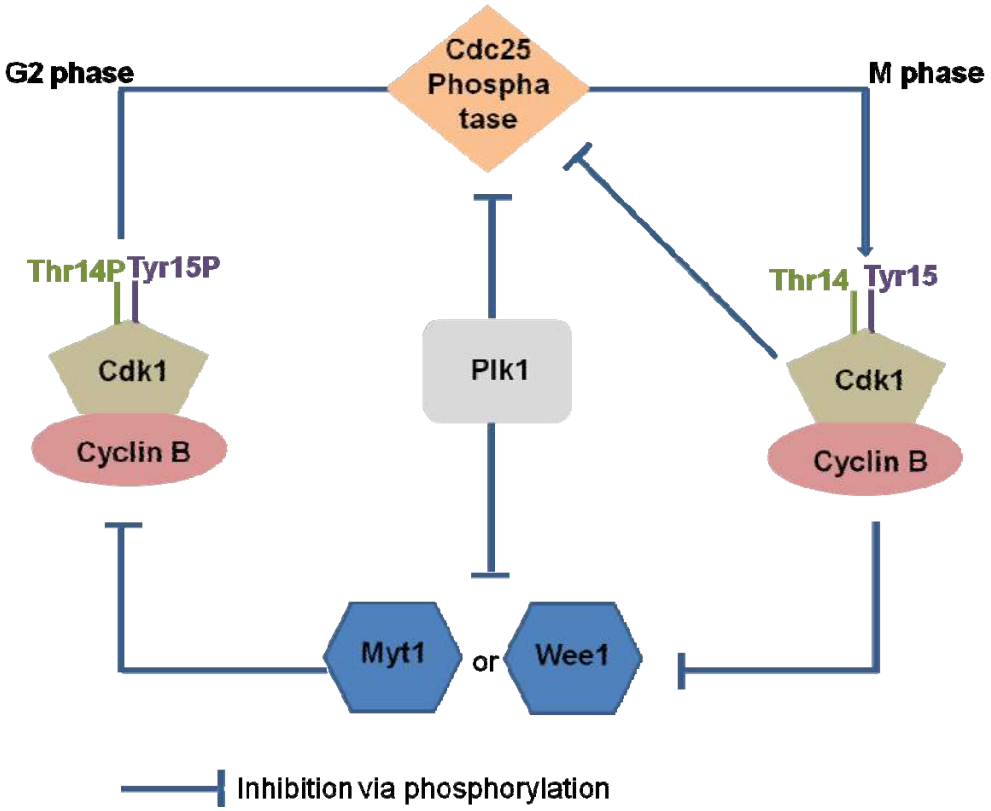

Fig 2. **Plk1 at G2/M transition.** Schematic Representation of involvement of Plk1 during G2/M phase by activating Cdk1/cyclin B by phosphorylating (inhibiting) Myt 1 and Cdc25 phosphatase (once Cdk1/cyclin B is activated)

Several lines of evidences have indicated that Plk1 phosphorylates Myt1[25] and cdc25[26]. *Xenopus* Plx1 isolated from egg extracts was shown to phosphorylate Cdc25 phosphatase and in turn regulating cylinB-cdk1 activity[26]. Also a recent study shows the phosphorylation of Cdc25 at serine 198 residue (Ser 198) which lies in the nuclear export signal and this phosphorylation causes nuclear translocation of cdc25 during prophase which might contribute to the activation of cdk1[27].
However the question arises how Plk1 is activated at the onset of G2/M phase. As mentioned earlier the polo box domain (PBD) of Plk1 adopts an inactive folded confirmation, in a manner that it interacts with the kinase domain and suppresses its activity. Recent advances have shown that Bora a Plk1 interacting protein peaks at the G2 phase and binds to Plk1 which releases the autoinhibition caused by PBD and make the T-loop accessible for the phopshorylation by Aurora A kinase at Thr210[28]. Once Plk1 is active it phosphorylates Bora at Serine at 497 and threonine at 501 (in a sequence of DSGYNT), this phosphorylation mediates the interaction of Bora with β-TrCP (which act as adapter between the substrate and ubiquitin ligase) and directs its proteasomal degradation[29]. Thus Aurora A and Bora cooperatively activates Plk1 and promotes Mitotic entry.

In response to DNA damage a checkpoint is activated which arrest the cells in G2 phase and prevents the activation of Cyclin B-Cdk1 complex. Various studies have reported that during checkpoint activation ATM (Ataxia telangiectasia mutated, a serine/threonine kinase**)** activates Chk1/Cds1 which further phosphorylates Wee1 and cdc25 which activate and inactivate them respectively. Studies done using DNA damaging agent adriamycin have shown that Plk1 inhibition occur during DNA damage checkpoint. Further over-riding the checkpoint with caffeine which inhibits ATM is shown to prevent the inhibition of Plk1 suggesting Plk1 is a target of DNA damaging checkpoint[30]. However, one study shows that when cells recover from the G2 DNA

damage arrest, they rely on a single pathway in which Plk1 mediates the degradation of Wee1 by phosphorylating the same[31].

Taken together these studies suggest that Plk1 plays a crucial role for Mitotic entry in cells undergoing normal cell division and cells recovering from the DNA damage checkpoint.

Role of Plk1 in Chromosome segregation

Duplicated chromosomes known as sister chromatids attach to the mitotic spindle via kinetochores in a bipolar fashion during metaphase. Chromosome segregation during anaphase involves two major processes: firstly the bipolar attachment of chromosomes and secondly the activation of APC/C complex and in turn spindle checkpoint silencing. Plk1 localizes at the kinetochores and are involved in kinetochore-spindle interaction and interaction with spindle checkpoint proteins. Studies have shown that cells depleted in Plk1 lacks focussed poles and are not able to generate pole ward pulling forces suggesting Plk1 plays an essential role in chromosome segregation[32].

Cohesion protein complex (comprising of four subunit namely Scc1, Scc3, Smc1 and Smc3) glue the sister chromatids together at the chromosome arm and centromeric region. Chromosome arm cohesion is removed during prophase for chromosome duplication whereas centromeric cohesion is degraded during metaphase to anaphase transition. Centromeric cohesin is protected by Shugoshin (Sgo1) family of proteins which are directed to the centromeres via interaction with protein phospahatase 2A (PP2A) in a Bub1 (budding uninhibited by benzimidazole) dependent manner[33]. In *Drosophila* POLO kinase phosphorylates the MEI-S332 (Sgo1 family protein) and removes it from the centromeric cohesions, however this removal of MEI-S332 do not accelerates chromosome segregation but may be essential for making cohesin protein more accessible[34]. However in another study, in vertebrates Plk1 directs the Sgo1 splice variant sSgo1 to the centrosomes during prophase and to centrioles during M phase and helps in maintaining integrity of spindle pole[35]. Plk1 also enhance the cleavage of cohesin subunit Scc1 by separase which otherwise remains inhibited by the interaction of securin. Securin is degraded by ubiquitin protein ligase called anaphase promoting complex at the onset of anaphase. Plk1 phosphorylates cohesin at the serine residue adjacent to Scc1 cleavage site and enhances its degradation, thus regulating sister chromatid separation[36]. During mitosis Plk1 profoundly localizes at the Kinetochore region which is occupied by spindle checkpoint proteins MAD (mitotic-arrest deficient) genes MAD1, MAD2 and MAD3 (BUBR1 in humans) and the BUB (budding uninhibited by benzimidazole) gene BUB1. So the question arises what is the role of Plk1 at kinetochores and how it is recruited at that site.

Several lines of evidence have shown that inner-kinetochore component INCENP and the spindle checkpoint protein Bub1 recruits Plk1 at the mitotic kinetochores[37, 38]. A parallel study has also identified that Plk1 phosphorylates PBD-binding protein, PBIP1 at Threonine 78 (T78) residue and self regulates the timing of its recruitment at interphase and mitotic kinetochores and promote proper chromosome segregation[39]. Plk1 also phosphorylates spindle checkpoint protein BubR1. Once BubR1 protein is phosphorylated at threonine 620 by Cdk1, it recruits Plk1 at the kinetochores which in turn phosphorylates BubR1 at Serine 676 position and helps in maintaining kinetochore-microtubule interaction (KT-MT) [40]. Plk1 also helps in maintaining proper microtubule dynamics during chromosome segregation by phosphorylating mitotic centromere-associated kinesin (MCAK). Plk1 interacts and phosphorylates six serine residues in the C-terminal of MCAK which in turn activates the microtubule depolymerising activity of MCAK and thus promoting chromosome segregation[41].

At the kinetochores, Plk1 not only stabilizes kinetochore-microtubule interaction (KT-MT) but also corrects KT-MT attachment errors. This was proved in a study where Plk1 phosphorylates kinesin-13 protein Kif2b at two residue namely threonine 125 (T125) and Serine 204 (S204).

Phosphorylation at S204 promotes Kif2b localization at kinetochore whereas phosphorylation atT125 promotes Kif2b activity for correcting any KT-MT attachment errors[42]. Plk1 also interacts and modulates component of spindle checkpoint signalling example of which is PICH (PLK1-interacting checkpoint "helicase"). Once Cdk1 phosphorylates PICH at threonine 1063 it further interacts with Plk1 which phosphorylates PICH and recruits it at the kinetochores where it forms an essential component of spindle checkpoint signalling[43].

Intriguingly, these studies suggest that Plk1 plays an essential role in chromosome segregation and microtubule-kinetochore interaction.

Role of Plk1 in Cytokinesis

In animals, cytokinesis begins after the segregation of sister chromatids in anaphase where actomyosin contractile ring is formed at the equatorial cortex which leads to the formation of cleavage furrow and its ingression divides the cytoplasm and gives rise to two daughter cells.

Polo like kinase involvement in cytokinesis was first reported in yeast *Schizosaccharomyces pombe*. The mutation in Plo1 (homolog of Plk1 in yeast) leads to defect in septum formation and nuclear division[4].

At the onset of cytokinesis, bipolar spindle reorganizes where kinesin motor proteins and microtubule binding and bundling proteins binds to the plus end of the microtubules and give rise to spindle midzone or central spindle. Central spindle plays a crucial role during cytokinesis as it marks the position of cleavage furrow. Central spindle comprises of centralspindlin complex which is formed by a kinesin like protein, Mitotic kinesin-like protein 1 (MKLP1) and Rho GTPase activating protein (Rho-GAP), CYK4[44, 45].

Polo like kinase Plk1 prevents the premature formation of spindle midzone by phopshorylating at threonine 602 residue of Protein Regulator of Cytokinesis (PRC1) and thus negatively regulating its microtubule binding and bundling activity[46]. Plk1 localizes itself at the spindle midzone by phosphorylating mitotic kinesin-like protein 2 (MKLP2) at Serine 528 and also by interacting with microtubules[47]. Recent studies using the advantage of chemical genetics have shown the role of Plk1 upstream of RhoA signalling for generating cleavage furrow. In this study authors have PLK1-null human retinal pigment epithelial (RPE) cells which are being complemented by a genetically modified mutant Plk1 allele. Modification is done in the manner that catalytic pocket of Plk1 is enlarged to accept bulky purine analogs as ATPcompetitive inhibitors (Plk1as, for analog-sensitive) [48]. This study highlighted that Plk1 interacts and phosphorylates at Serine 157 site of the Rho-GAP subunit of centralspindlin i.e. HsCYK-4 (also known as MgcRacGAP) at the midzone. This phosphorylation at S157 creates a docking site for the tandem BRCT repeats of the Rho GTP exchange factor Ect2. This recruitement of Ect2 (Rho GTP) via HsCYK4 phosphorylation leads to activation of RhoA- GTP which leads to actin polymerization and myosin II motor activity at the spindle midzone leading to cleavage furrow formation and ingression [49, 50, 51].

In totality the power of chemical genetics revealed the role of Plk1 in a temporally and spatially controlled manner during cytokinesis.

Conclusion

Plk1 plays an important role during various stages of cell cycle where it interacts with different substrates and targets its localization or degradation. However the list of substrate of Plk1 is not yet complete and it would be interesting to analyse how it regulates other mitotic proteins. Also speculating the role of Plk1 during differentiation would be fascinating. Alteration in the level of

Plk1 (either over expression or down regulation) is associated with tumor development[52]; therefore it could also serve as a prognostic marker for many cancers.

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Protoplasts – an important tool in Rice Biotechnology

V. Jisha and Rajeshwari Ramanan

Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad-500007.
raji@ccmb.res.in

BACKGROUND

Plant protoplasts have been used as a transient assay system similar to the mammalian tissue culture lines, for rapid and high-throughput analysis of gene functions in plants. Plant protoplasts show physiological perceptions and responses to hormones, metabolites, environmental signals and pathogen derived elicitors, similar to cell autonomous responses in intact tissues and plants. Protoplast transient expression systems have greatly contributed towards elucidating plant signal transduction pathways in combination with genetic, genomic and transgenic approaches. There are many advantages of using fresh tissues as protoplast sources as compared to cell culture lines. Protoplasts represent active and homogenous cell populations that retain their cell identity and differentiated state, show high transformation efficiency with low maintenance and can be isolated from fresh tissues and used immediately for experiments without the time consuming step of cell culture establishment. Most of the quantitative and physiological responses can be observed and measured within 2-10 hours after DNA transfection, the experiments do not require sterile techniques and complex culture medium thus saving lot of time and cost. Proteins that are normally expressed in other cell types and tissues or cause lethality when deleted or over expressed can be ectopically expressed and examined in protoplasts to learn their molecular and cellular functions. However, there are some limitations too with the protoplast system. Since they are very fragile without the cell wall, extreme care and standardization is required to get consistently intact protoplast preparations and high transfection efficiencies. Studies related to cell wall functions, plasmodesmata and cell-cell interactions cannot be addressed.

Protoplasts from several plant species including *Arabidopsis thaliana* (Ath), tobacco, maize, barley have been successfully used for transient assays following polyethylene glycol (PEG)-mediated transfections with plasmids (Jiang et al., 2013). Most common sources of protoplasts are leaves, stem, calli or suspension cell cultures but other plant tissues have also been used to analyze tissue-specific gene expression and processes. Protoplasts isolated from rice endosperms did not show autoflourescence at 490nm and could be useful tool for studying mechanisms of seed development and quality (Takahashi et al., 2004). Protoplasts isolated from petals of *Petunia hybrid*a could be easily distinguished from mesophyll-derived protoplasts by their color and showed highly specific promoter activity and protein trafficking (Faraco et al., 2011). Proteomics of protoplasts isolated from root epidermal cells of GL2 pro-GFP (GFP expression driven by epidermal-specific promoter) expressing transgenic plants by fluorescence-activated cell sorting, identified several low abundant proteins in various Ath root cell layers (Fukao et al., 2013).

Rice is the staple food for a large proportion of the Indian population. The availability of genome sequence, global gene expression profiles, various types of mutant collections and standardized methods of transformation and RNAi, have made rice a model system for monocots and generated a wealth of resources for functional genomics. A large-scale analysis of gene functions can be

facilitated by developing homologous rice protoplast transient assay system (RPTAS). An improved method to isolate a large number of protoplasts from stem and sheath tissues of both young and mature rice plants, etiolated and green rice tissues have been reported recently (Bart et al, 2006, Chen et al 2006, Zhang et al., 2011). In our lab, the following method for isolation and transfection of rice protoplast works best.

Isolation of protoplasts from rice green tissue

The growth conditions and seedling stage were found to be critical for protoplast isolation. Seeds of rice cultivar TN-1 were surface sterilized with 2.5% sodium hypochlorite for 15 minutes followed by 5-6 times washes with sterile water. Seeds were kept in petriplates in the growth chamber with 12hr light/dark cycle for 5-6 days. Seedlings were then transferred to normal soil in pots in green house and 8-10 day old rice seedlings were used for protoplast isolation. Green tissues from the stem and sheath of 40-60 rice seedlings were used. A bundle of rice plants (about 30 seedlings) were cut together into approximately 0.5 mm strips using sharp razors. The strips were immediately transferred into 0.6 M mannitol for 10 min in the dark. After discarding the mannitol, the strips were incubated in an enzyme solution (1.5% Cellulase RS, 0.75% Macerozyme R-10, 0.6 M mannitol, 10 mM MES at pH 5.7, 10 mM CaCl2 and 0.1%BSA) for 4-5 h in the dark with gentle shaking (60-80rpm). After the enzymatic digestion, an equal volume of W5 solution (154 mM NaCl, 125 mM CaCl2, 5 mM KCl and 2 mM MES at pH 5.7) was added, followed by vigorous shaking by hand for 10 sec. Protoplasts were released by filtering through 40 μm nylon meshes into round bottom tubes with 3-5 washes of the strips using W5 solution. The pellets were collected by centrifugation at 1,500 rpm for 3 min with a swinging bucket rotor. After washing once with W5 solution, the pellets were then resuspended in MMG solution (0.4 M mannitol, 15 mM $MgCl_2$ and 4 mM MES at pH 5.7) at a concentration of 2×10^6 cells mL^{-1}, determined by using a haemocytometer. All procedures were done at room temperature. A preparation of intact protoplasts is shown in Fig 1.

PEG-mediated protoplast transfections

Maximum PEG-mediated transfection efficiencies of 53-75% with smaller plasmids of 4.5 kb, 45-66% with large-size 13 kb plasmids and up to 45% co-transfection efficiencies with two 13 kb plasmids have been reported in rice green protoplasts (Zhang et al., 2011). Briefly, for each sample 5-10 μg of plasmid DNA were mixed with 100 μL protoplasts (about 2×10^5 cells). For co-expression assays, the total plasmid DNA was between 10 μg and 15 μg. 110 μl freshly prepared PEG solution [40% (W/V) PEG 4000; 0.2 M mannitol and 0.1 M $CaCl_2$] were added, and the mixture was incubated at room temperature for 10-20 min in the dark. After

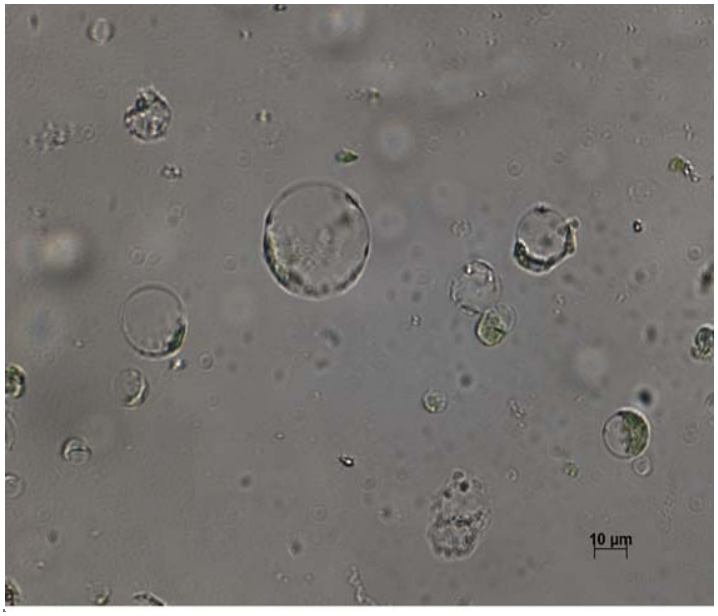

Figure 1. Intact protoplasts from 8 day old rice seedlings. Image was taken under an axioplan microscope with 40x objective

incubation, 1.1ml W5 solution was added slowly. The resulting solution was mixed well by gently inverting the tube, and the protoplasts were pelleted by centrifugation at 1,200 rpm for 3 min. Supernatant was discarded and pellets were washed once more with W5 solution. The protoplasts were resuspended gently in 50µl of WI solution (0.5 M mannitol, 20 mM KCl and 4 mM MES at pH 5.7) for microscopy. The protoplasts were incubated under dark at room temperature for 6-16 h and observed using a confocal laser scanning microscope.

APPLICATIONS OF RPTAS

RPTAS has been extensively used in gene expression studies to dissect the functions of cis-elements and trans-factors and identify key regulators in many essential processes and signaling pathways involved in plant physiology, immunity, growth and development. Various reporter systems such as LUC [luciferase] and GUS [β-glucuronidase] have been used for the quantitative measurement of gene expression. Glucocorticoid-inducible luciferase reporter gene, dual RPTAS harboring tandem copies of codon of interest at 5'-end has been described to study translation efficiencies in rice (Hamamoto et al., 2013).

RPTAS has been used to decipher phytohormone, light and pathogen-induced signaling pathways. RPTAS was used to study the regulation of Abscisic acid (ABA)-inducible wheat promoter of a gene encoding a major embryo protein, using GUS as reporter and a several-fold increase in GUS activity within 1 h of ABA treatment by 165 bp promoter region indicated regulation of the gene transcription by ABA (Marcotte et al., 1988). The role of Ath bZIP transcription factor required for ABA-response in seed and vegetative tissues in ABA signaling pathway was demonstrated by its transactivation of ABA-inducible wheat *Em*, Ath *AtEm6*, bean β-phaseolin and barley *HVA1* and *HVA22* promoters in rice protoplasts (Gampala et al., 2002). RPTAS was used to show interaction between GIBBERELLIN INSENSITIVE DWARF 1 with SLENDER RICE 1 only in the presence of GA but not other plant hormones (Fujikawa et al., 2014). Protoplasts from green tissues are photosynthetically active as shown by increase in transcript levels of endogenous photosynthetic genes and their downregulation upon treatment with norflurazon (a retrograde plastid signaling

inducer) when Gfp-tagged light–related transcription factor OsGLK1 was transiently expressed in protoplast (Zhang et al., 2011).

Functional analysis of defense genes in response to elicitors from bacterial and fungal pathogens has been carried out using RPTAS. Rice resistance gene Xa21 is a pathogen recognition receptor (PRR) encoding for a LRR-kinase protein which recognizes pathogen associated molecular pattern (PAMP), a sulphated peptide(AxY^S22)secreted by the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo). Cleavage of plasma membrane-localized Xa21-GFP in response to biologically active AxY^S22 from Xoo and translocation of the intracellular kinase domain which carries a nuclear localization signal (NLS), to the nucleus was demonstrated using RPTAS (Park and Ronald, 2012). Monitoring expression of defense genes such as *PBZ1* and *ChitinaseIII* in response to elicitor from cell wall extracts of blast fungus *Magnaporthe grisea* by fusing their promoters to firefly luciferase reporter gene was done in protoplasts isolated from young as well as mature rice stem and sheath tissues by checking for luciferase activity relative to activity of Gus gene driven by Ubi-promoter in co-transfected protoplasts indicating that RPTAS is also suitable for analysis of developmentally regulated genes (Chen et al., 2006). Dissection of protein domains required for interaction of a negative regulator NRR with AthNPR1, a master regulator of systemic acquired resistance (SAR) and its inability to repress NPR1 function upon mutating the domain was demonstrated using RPTAS (Chern et al., 2012). RPTAS was used for *in vivo* fluorescence resonance energy transfer (FRET) analysis to study regulation of the interaction between OsRac1 (a small GTPase Rac involved in resistance to rice pathogens by enhancing PAMP-induced reactive oxygen species) and OsRboh (respiratory burst oxidase homolog) by cytosolic Ca^{2+} concentration (Wong et al., 2007).

Protein-protein interactions using bimolecular fluorescence complementation (BiFC) and firefly luciferase complementation (FLC) of split reporter proteins and protein co-immunoprecipitations (co-IP) have been analysed successfully using rice mesophyll protoplasts (Yang et al., 2013) . BiFC was used to show dimerization of bZIP transcription factor in rice protoplasts and co-expression of 2-Cys peroxiredoxin BAS1-cmyc-YFP^N and thioredoxin OsTRX m5-HA-YFPC constructs in rice protoplasts showed YFP signal only in chloroplast suggesting that chloroplast localized BAS1 is a potential target of OsTRXm5 whereas FLC was used to confirm known interaction between SGRT1 and RAR1(important components of disease resistance signalling in higher plants) with only SGT1a-NLuc and CLuc-RAR1 constructs showing strong luciferase activity relative to Renilla luciferase activity used as internal control (Zhang et al., 2011). Protein-protein interactions of 100 proteins involved in bacterial, systemic acquired resistance and submergence signalling pathways were validated by co-expression of transcripts in rice protoplasts using BiFC of split YFP and the interactome analysis supported evidence of cross talk between biotic and abiotic stress responses (Seo et al., 2011). A high throughput method using FLC in 96-well plates has been developed to detect regulated protein-protein interactions towards establishment of rice interactome (Fujikawa et al., 2014). FRET, co-localizations and BiFC in rice protoplasts were used to identify OsCEBiP, OsCERK1, OsRacGEF1, and OsRac1 as key components of ‘defensome’ involved in fungal-chitin driven rice immunity (Akamatsu et al., 2013).

RPTAS is suitable tool for sub-cellular localizations of rice proteins, which would help in the identification of their functions. Fluorescent organelle-specific markers based on well established targeting sequences have been developed in Ath as indicators for endoplasmic reticulum (ER), golgi bodies, peroxisomes, plasma membrane (PM), mitochondria and plastids (Nelson et al., 2007) and majority of these were found to target correct compartments in rice protoplast too (Zhang et al., 2011). We have also used ER-YFP, Golgi-YFP and PM-mCherry markers to show organelle-specific

localizations to endoplasmic reticulum, golgi and plasma membrane respectively, in rice protoplasts (Figure 2).

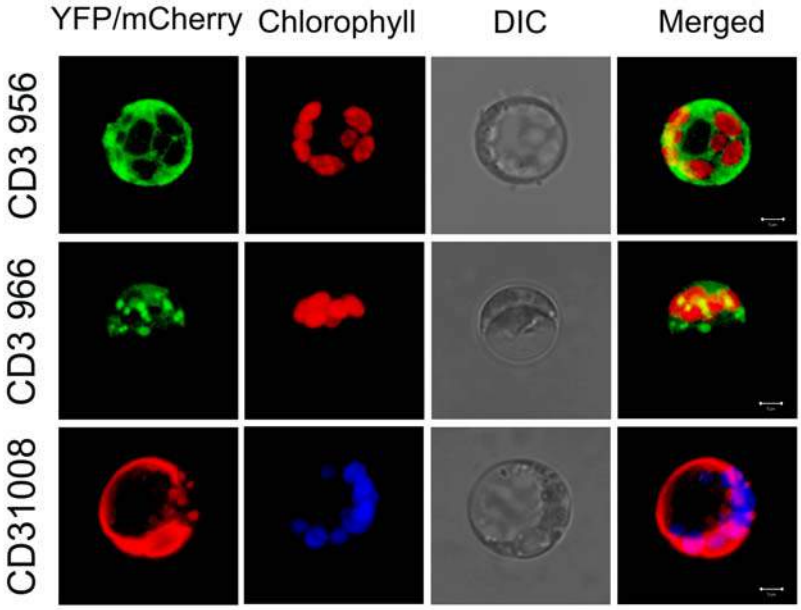

Figure 2. Subcellular localization of Arabidopsis organelle markers in rice protoplasts. [CD3 956-ER-yfp (green), CD3 966-golgi-yfp (green), CD3 1008-Plasma membrane-mCherry (red)]. Chlorophyll autofluorescence is in red in lanes 1&2 or blue in lane 3.

We expressed three rice proteins OsXb22a, OsXb22b and OsEREBP1 as GFP-fusions in rice protoplast to determine their sub-cellular localizations. OsXb22a, which has a predicted chloroplast signal, was found to co-localize with chloroplast autofluorescence indicating its plastid localization (Figure 3a). OsXb22b, an isoform of OsXb22a lacking the chloroplast signal was distributed in the cytosol as fluorescent spots resembling golgi bodies (Fig. 3b). OsEREBP1, an AP-2 domain containing transcription factor was also localized in chloroplast and appeared as punctuate structures in red autofluorescing region of the chloroplast (Fig. 3c).

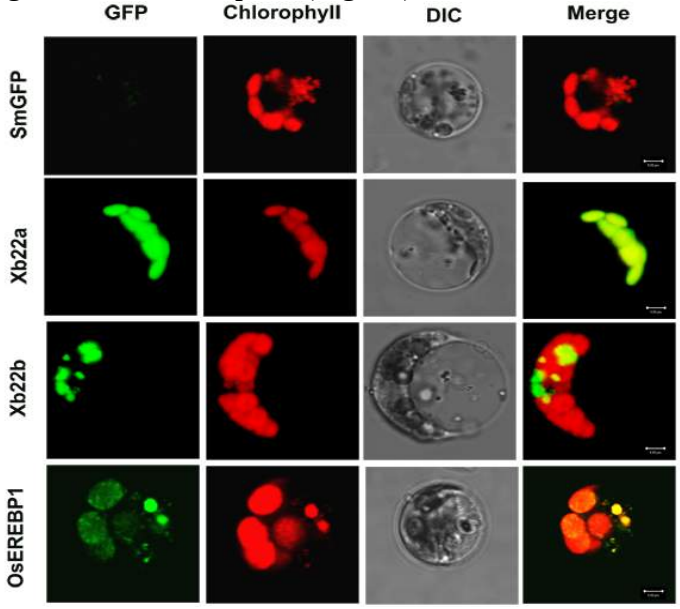

Figure 3. Subcellular localization of OsEREBP1-SmGFP, Xb22a-SmGFP and Xb22b-SmGFP fusion proteins.

Use of short interfering RNA (siRNA) in a concentration dependent manner to silence luciferase gene in rice protoplasts expressing high levels of luciferase activity opens up new opportunities to study gene functions whose deletion cause cell lethality (Bart et al., 2006). RPTAS has been used for siRNA silencing of exogenous *GFP* and endogenous spot leaf 1 (*SPl1*) gene involved in compatible and incompatible rice blast interactions (Chen et al., 2012). RPTAS have been used as a valuable tool to quickly screen transcription activator like effector-nucleases (TALENs) for targeted knockout mutations in genes for their subsequent use in rice transformation (Shan et al., 2013).

In conclusion, plant protoplasts are an important tool for addressing problems in plant cell biology, similar to the animal and human cell-based assay systems. Protoplasts from rice provide a versatile transient assay system to analyze gene functions on a large-scale and can be used to dissect signaling pathways in various physiological processes by over expression, silencing or targeted mutations of genes.

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HIGH RESOLUTION IMAGING SYSTEMS ; CONFOCAL MICROSCOPY AND BEYOND

NANDINI RANGARAJ
Centre for Cellular and Molecular Biology,
Uppal Road, Hyderabad-500007
nandini@ccmb.res.in

History

The invention of the fluorescence microscope in 1904, the introduction of fluorescently labelled antibodies in 1941 and the realization that antibodies to normal proteins such as actin and tubulin could be raised (Lazarides and Weber, 1974) marked the beginning of imaging as a tool in the late 1970'S and 1980's. Around this period fluorescent stains to monitor intracellular parameters such as calcium ion concentration also came into use (Tsein et al., 1985) and a large proportion of the work in biology relied on fluorescence microscopy. Soon the limitation of this technique also became obvious, in that, the out of focus parts of a thick biological specimen gave rise to a uniform glow against which fine detail could not be distinguished.

The Confocal microscope was invented by Minsky in 1955 (Minsky, 1988).The word confocal used by Brakenhoff (Brakenhoff et al., 1979) means a microscope in which the illumination is confined to a diffraction limited spot in the specimen and the detection is similarly confined by placing an aperture in front of the detector in a position optically conjugate to the focussed spot. The result of this arrangement is that the response of the instrument to a fluorescent point object falls off approximately according to an inverse fourth power rule with the distance from the plane of focus. This produces an optical sectioning effect in which the glare from out of focus region is almost completely eliminated.

Since the confocal views only a single point in the specimen at one time, special apparatus was needed to build up a 2-dimensional image by scanning the illuminated spot over the specimen. Minsky's prototype and the system of Brakenhoff (Brakenhoff et al., 1985) relied on moving the entire specimen and its supporting stage in two directional raster fashion relative to stationary optics. By this, axial series of images of labelled nuclei was shown with clarity. But these microscopes were too slow and vibration sensitive.

In the meantime an alternate approach to confocal microscope was adopted by Petran and colleagues (Petran et al., 1968) where the specimen was illuminated with many spots of light by placing a Nipkow disc on the image plane, in effect many thousands of confocals working in parallel. The disk was spun to give a continuous image and this worked for reflective images but attenuated fluorescent images badly.

The idea of moving the laser beam and keeping the specimen stationary was developed by White and Amos (White and Amos, 1987; Amos and White, 2003). They used two orthogonal galvo driven mirrors located in the aperture plane of microscope to give high scanning rate. This along with digital image capture was incorporated in their prototype in 1987 which later formed the basis for the first commercial confocal microscope by Bio-Rad.

The Principle

The major limitation of a conventional light microscope was that it gave only a 2D image. The biological specimen was 3D and to view it the specimen would have to be first physically sliced

into thin sections which introduced artifacts. Hence the confocal system was seen as a boon as it gave optical sections.

Confocal imaging systems are based on the principle that the illumination and detection systems are focused on the same volume element (voxel) of the specimen. The illuminated voxels are sampled in such a way that the signals from the voxels in the focal plane are detected and the signals from outside the plane of focus are removed by a confocal aperture (pinhole). Thus the illumination, specimen and detection (pinhole) are at the same focus i.e. they are in optically conjugate focal planes or confocal.

Confocal imaging requires that the optical system for excitation remains aligned with that for detection so that both are focused on a single point in the specimen. This is achieved if one uses epi- rather than trans- illumination, where the light for both illumination and recording passes through the same objective lens and also through the same part of the specimen. Due to the phenomenon of diffraction of light, the spot focussed in the specimen is not actually a point but an Airy disk having a size inversely proportional to the numerical aperture (NA) of the objective and of the wavelength of light (λ). This airy pattern influences the lateral resolution. Resolution can be defined as the minimum distance between two objects at which a certain contrast is achieved. As two point objects (which are actually airy disks) come closer, the centre-to-centre distance between the two objects at which a certain contrast is achieved is called the contrast cut-off distance and it is this distance which can be resolved in the image. It is this cut-off distance that is smaller in a confocal microscope as compared to wide-field microscope and has a better lateral resolution(X-Yresol) (Pawley and Centoze, 1998; Stelzar, 1998). Figure1 depicts the image clarity of actin cytoskeleton taken with a confocal microscope as against a regular fluorescence microscope. Every 2D image shows the location of fluorescent features in a single optical section and using this, it is relatively easy to build up a 3D image by collecting data from adjacent focal planes i.e. along the Z-axis of the specimen that is reasonably transparent (Fig2).

Other Techniques

The other method for obtaining 3D intensity data from an intact biological specimen is referred to as wide field method or optigrid method. It uses a cooled charged coupled device (CCD) as image detector or an optigrid in the illumination path attached to a conventional epifluorescence microscope or both to acquire sequentially a set of extremely precise images at adjacent focal planes throughout the volume of interest. In this case each image contains data from both in focus and out-of-focus planes. Deconvolution algorithms are used to remove the effects of data from the out of focus planes to produce images that contain only in focus data (Agard et al., 1989). The use of an optigrid in the illumination path further assists in distinguishing data from in focus and out of focus planes by capturing 3 images at each focal plane with different grid positions (Wilson et al., 1998). It should be noted that this kind of non-confocal optical sectioning using algorithms requires accurate knowledge of the point spread function (psf) of the objective lens in use.

In 1990 Denk etal (Denk et al., 1990) showed that using pulsed lasers it is possible to obtain sufficient peak power intensity levels in a focussed spot to cause multi photon absorption. With a sufficient long excitation wavelength and increasing the illumination intensity by a factor of 10^5 or more it is possible for a given dye to absorb 2-3 photons simultaneously. As multiphoton absorption is proportional to the square of the excitation intensity, the adjacent focal planes are not subjected to intense light and therefore fluorescence emission is generated only in a single plane of the specimen, eliminating the need for a confocal pinhole. The advantages of multiphoton are that infra red (IR)

photons are less damaging to biological tissue and have greater penetration into the specimen. As the Stokes shift between excitation and emission wavelength is much greater when IR is used, it is possible to design filter sets that use emitted light much more efficiently than those with single photon excitation.

Applications

The invention of confocal microscopy therefore saw a lot of applications.

Considerable information was gained from single optical sections of multi-labelled samples and helped to identify sub-cellular structures in 3D. For example restricted localization of pC3G a phosphorylated form of Rap1GNEF (Guanine nucleotide exchange factor) was evident from confocal sectioning of mammalian Cos-1 cells expressing the kinase c-Abl. A 3-D reconstruction of the same revealed that areas showing pC3G did not necessarily correspond to the sites of cell attachment as seen from the view of the adherent surface. In the same study fluorescence intensity quantitation profiles showed that the activation of the kinase c-Abl coincided with the phosphorylation of its substrate C3G (Mitra and Radha, 2010).

Confocal also provides information on colocalisation of two or more structures. Studies on colocalisation of optineurin a multifunctional protein or its disease causing mutant with their interacting partners such as transferrin receptor (TfR), Rab8 or Rab11 have helped in elucidating their role in endocytic trafficking of transferrin receptor in mammalian cells (Nagbhushana et al., 2010). With the advent of genetically expressed fluorescent proteins, confocal studies could be carried out in live specimens. Live cell confocal studies of the vesicles formed by green fluorescent protein (GFP) tagged optineurin and its mutants and the analysis of their size, diffusion and dynamics have supported their role in the endocytic trafficking process (Nagbhushana et al., 2010). In another study, Sirohi et al examined the autophagic flux using the mCherry-GFP-LC3B. MCherry-GFP-LC3B is detectable in autophagosomes and also in autolysosomes but GFP fluorescence is seen only in autophagosomes and not in autolysosomes due to sensitivity of GFP to low pH in autolysosomes. Such studies helped in elucidating the role played by M98K, an optineurin mutant in enhancing autophagic cell death in retinal cells. (Sirohi et al., 2013).

Confocal imaging followed by the multiphoton microscopy, various illumination and detecting techniques and use of algorithms, helped to study pathways and events in living or fixed cells and tissues with greater resolution and clarity. This was possible because such techniques were compatible with live samples and were minimally invasive. However spatial resolution of ~200nm and z-resolution of ~600nm was still a limitation with the techniques mentioned so far. It was in the mid 1990's that the imaging field broke this diffraction limit barrier. It gave way to the development of super resolution imaging techniques.

Super Resolution Microscopy

Many researchers working towards breaking the diffraction limit realised that separation of tiny fluorescent objects that were less than 200nm apart was possible by switching their signals on and off sequentially so that they can be seen consequetively.To switch a fluorescent molecule on and off requires two states connected by a transition, representing the actual switch. Switching distinguishes the objects, but to assemble an image their coordinates were also needed. Therefore either the coordinates in the sample were targeted by using optical methods to actively define the area in the sample where the fluorophore must be on or off or individual fluorophores were allowed to go on and off stochastically and later the coordinates, where the events occurred were determined. Techniques such as Stimulated Emission Depletion (STED) (Hell and Wichmann, 1994), Ground State Depletion (GSD) (Hell and Kroug, 1995) and Structured Illumination Microscopy (SIM) (Gustafsson, 2000) follow the optical route involving an ensemble of molecules whereas

Photoactivated localization microscopy (PALM) (Betzig et al., 2006; Hess et al., 2006) and Stochastic optical reconstruction microscopy (STORM) (Rust et al., 2006) follow single molecules through the stochastic route. The principle and resolution capabilities of each of these scopes are given in Table1.

STED was the first far field super resolution imaging technique to be applied to cell imaging (Hell and Wichmann, 1994). It uses spatially modulated and saturable transitions between two molecular states. Specifically the sample is illuminated by two laser beams - an excitation laser pulse is immediately followed by a red shifted pulse called the STED beam. Excited fluorophores exposed to the STED beam are almost instantly transferred back to their ground states by means of stimulated emission. This non-linear (nearly exponential) deexcitation of the fluorescent state by the STED beam is the basis of breaking the diffraction limit. The STED pulse is modified to feature a zero-intensity point at the focal centre and strong intensities at the spot periphery creating a doughnut shape. If the two pulses are superimposed, molecules that are close to the zero of the STED beam are allowed to fluoresce, thus confining the emission to the same spot. STED was conceived in the context of laser scanning microscopy and was designed to directly minimize the size of the scanned focal point. This technique has achieved a resolution of ~45nm.An alternate approach is the concept of SIM (Gustafsson, 2000). If two patterns are superposed multiplicatively, a beat pattern called moiré fringes will appear in their product. In this case one of the patterns superimposed is the unknown sample structure and the other pattern is the structured excitation light intensity. If the illumination pattern is known, the moiré fringes will contain the information about the unknown structure. Thus one can gain access to high resolution information about the sample by observing its appearance under carefully controlled illumination patterns. The lateral resolution observed in this case is ~70nm. With 3D- SIM an approximate two fold increase in axial resolution can be achieved by generating an excitation light modulation along Z-axis using three-beam interference (Gustafsson et al., 2008).

The basic principle behind PALM and STORM is that the position of a single molecule can be localized to 10 nm accuracy or better if enough photons are collected and there are no other similarly emitting molecules within ~200 nm.These techniques determine the nanoscale localisation of individual fluorescent molecules by sequentially switching them on and off with light of different wavelengths. In each imaging cycle most molecules remain dark but a small percentage of molecules are stochastically switched on, imaged and localised. Repeating this process for many cycles allows the reconstruction of a super-resolution image with a lateral resolution of ~20 nm. (Betzig et al., 2006; Hess et al., 2006; Rust et al., 2006).

Conclusion

In the last three decades light microscopy has evolved from a compound microscope to the most sophisticated system rendering 3D information with high resolution in live samples. This has been possible due to the innovation in the field of optics, lasers, electronics, computers, imaging software and the development of new fluorescent dyes. However the sample preparation has not seen much of a change. Thus Cell Biologists can get a lot more information from a single sample preparation with the present day technology.

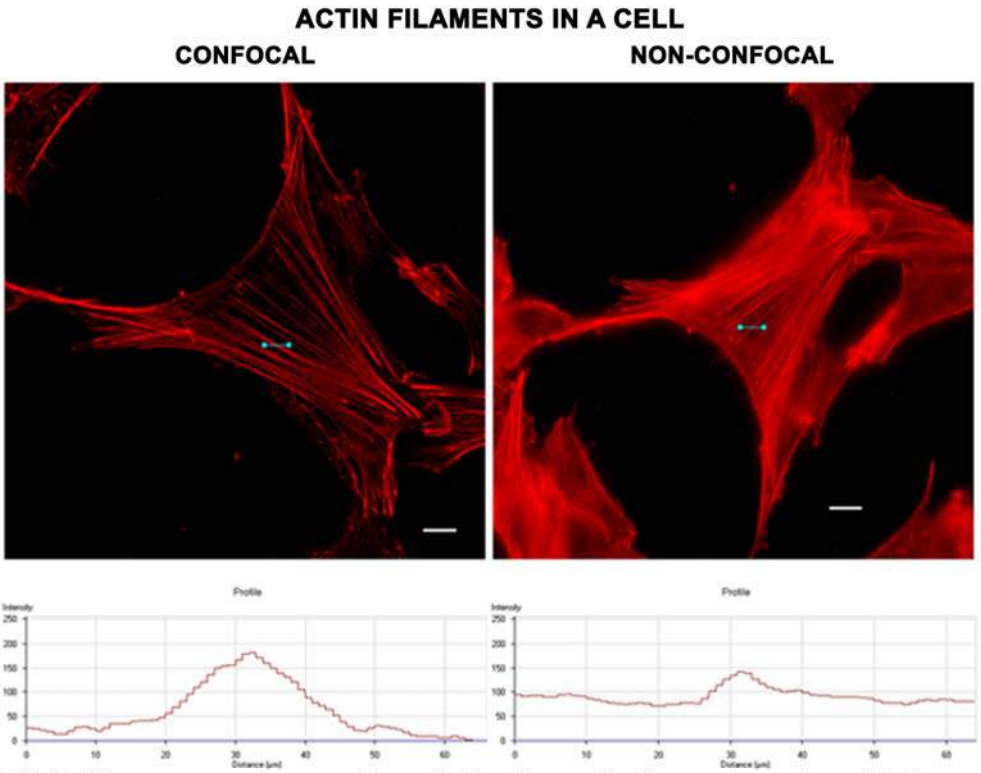

Fig1. Fluorescence image of a cell showing actin filaments captured in the confocal and non-confocal mode. Actin is stained with rhodamine phalloidin. Confocal image shows actin with greater clarity.
Intensity profile graphs given below the image depict that the non-confocal image has greater background due to out-of-focus fluorescence as compared to the confocal image. Bar=10um.

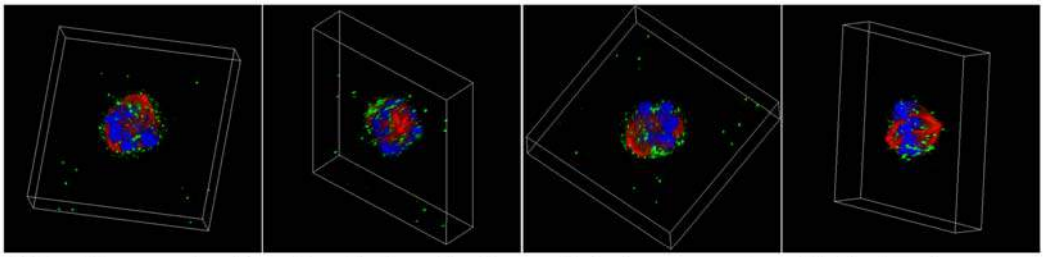

Fig2. 3D reconstruction of a mitotic cell using multiple images captured in the Z-axis. The microtubules have been stained for alpha tubulin shown in red and chromosomes are in blue. Staining in green is that of phosphorylated C3G which is dispersed in the cytoplasm during mitosis. The panel shows 3dimensional view of the cell from different angles.
Image courtesy: Dr.V.Radha.

PRINCIPLES, PROPERTIES AND APPLICATIONS OF CURRENT IMAGING TECHNIQUES

	Widefield + deconvolution	CLSM	3D SIM	STED	CW-STED	PALM / STORM
Principle	Computational image restoration improves widefield image z-stacks	Improves resolution by redesigning optical path using laser excitation and pinhole at detection	Periodic illumination pattern exciting the fluorescent samples gives more fringes in the emission distribution improving resolution	This uses non-lnear saturation process for controlled excitation of previously excited fluorophores giving high resolution	Same as STED but uses con-tinuous wave lasers for excitation and depletion so that conventional dyes can be used	Nanoscale localization of individual fluorescent molecules can be determined by sequentially switching them on and off. Repeating the process allows reconstruction of super-resolution image
Lateral Resolution X-Y	180-250 nm	180-250 nm	100-130 nm	~ 60 nm	~ 70 nm	~ 30 nm
Axial Resolution Z	500-700 nm	500-700 nm	~ 300 nm	~ 700 nm	~560 nm	140 nm
Temporal Resolution (512 x 512)	ms	ms-s	ms-s	ms-min	ms-min	s-min

	Widefield + deconvolution	CLSM	3D SIM	STED	CW-STED	PALM / STORM
Illumination emission dependence	Linear	Linear	Linear	Non-linear	Non-linear	Linear
Detectors	CCD/CMOS	PMT / APD	CCD/CMOS	PMT / APD	PMT / APD	CCD/CMOS
Advantages/ Disadvantages	All dyes can be used;live samples can be used; used for sparse samples such as filaments and vesicles; risk of arte-facts; PSF needs to be calculated	All dyes can be used; extensively used for different kinds of samples; pinhole needs to be closed to 1AU to see the effect and thus most of fluorescent light is discarded; diffraction limited optics	Standard dyes which are photostable can be used; used to study replication foci; mito-chondria; tubulin etc.; restricted use for live cells; reconstruct tion bears risk of artefacts	Dyes require special characteristics; used to study nuclear pore complex; restricted use for live cells, but have been used to study protein distri-bution and dynamics in membranes and vesicles	Some conventional dyes can be used; two color STED has been done; restricted use for live cell	Dyes require special characteristics; labelling density is critical; used for imaging particles and filaments; restricted use for live cells

Table: adapted from Schermelleh et al.

Foot Note:

CCD - Charge Coupled Device
CMOS - Complementary Metal-Oxide Semiconductor
PMT - Photomultiplier tube
APD - Avalanche photodiode
AU - Airy unit

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A REPORT ON 7th Asia-Pacific organization of Cell Biology (APOCB) Congress
Held from 24th to 27th February 2014 at Singapore

The four days congress organized by Dr Paul Matsudaira, National University of Singapore, held at Biopolis, Singapore, started with the Registration and three pre-meeting workshops on "Bioinformatics" conducted by Dr Omar Harb, University of Pennsylvania, USA, "Scientific paper and grant writing" conducted by Pernile Rorth, Institute of Molecular and Cell Biology, Singapore and "Ethics in Science" conducted by Kurt Albertine, University of Utah, USA on 24th February 2014. The scientific deliberations began in the afternoon with the welcome address of Dr Filipinas Natividad, President of APOCB and Dr Paul Matsudaira, Chairman of the 7th APOCB Congress. The opening session also contained two keynote presentations by Dr David Roos of University of Pennsylvania and by Dr K Vijay Raghavan, Secretary, Department of Biotechnology, New Delhi. A large number of Asian and other countries participated in the congress having nearly 300 delegates. It was most wonderful opportunity for some of the members of ISCB (Dr S Mayor, NCBS, Dr V Radha, CCMB and J K Roy, BHU) to represent our country in scientific sessions as well as to represent Indian Society of Cell Biology in the executive committee meeting of APOCB.

The second day had a plenary session on `Cell biology of host-pathogen interactions' which included 3 talks, 27 invited talks and 18 oral presentations in 9 concurrent sessions (3 parallel sessions at a time), viz., Cell biology of viral infections, Animal stem cell biology, Cancer cell biology, Parasite cell biology, Autophagy and cell death, Organelle biology, Cytoskeleton and cell cycle, Membrane trafficking, Omics; a poster session with 50 posters, a workshop on Electron microscopy conducted by FEI and the meeting of APOCB Executive Committee.

The third day had a plenary session on `Cell Biology of diseases' including 3 talks, 30 invited talks, 11 oral presentations and a panel discussion in 9 concurrent sessions (3 parallel sessions at a time), viz., Bacterial pathogenesis, Cell signaling, Cell biology education, Growth and polarity, Bioimaging-Electron microscopy, Evolutionary cell biology, Neurobiology, Bioimaging-Light microscopy, Microbial social behavior; a poster session with 50 posters and a workshop on electron microscopy conducted by JEOL.

The fourth day had 2 plenary sessions on `Stem cell and developmental biology' including 6 talks, 12 invited talks in 3 concurrent sessions, viz., Fungal pathogenesis, Cell biology of metabolism, Plant cells and development; a poster session with 60 posters and a workshop on microscopy conducted by Leica.

The scientific deliberations were of high standard and gave a chance of interacting with the leaders in different areas of Cell Biology. A list of the Plenary and invited talks is appended below to obtain an insight into the congress:

Plenary lectures:

Coronavirus- host cell interactions -*by Dr Dingxiang Liu (Singapore)* [Chair: Dr David Roos, USA]

A role for iNOS in plasma cell survival -*by Dr Anna George (India)*

Septin-mediatedplant infection by the rice blast fungus *Magnaporthe oryzae -by Dr Nicolas Talbot (UK)*

Metabolism and cancer therapeutics: targeting arginine addiction of cancers -*by Dr Hsing-Jien Kung (Taiwan)* [Chair: Dr Cheng-Wen Wu, Taiwan]

Lipid droplet fusion and obesity development -*by Dr Peng Li (China)*

Gap junction hemichannel medited vascular leak in the inflammatory response and cancer -*by Dr Colin Green (New Zealand)*

Systems biology of stem cells -*by Dr Ng Huck Hui (Singapore)* [Chair: Dr Philip Ingham, Singapore]

Plant stem cells –*by Dr David Jackson (USA)*

Intracellular Ca^{2+} dynamics shapes the neural tube -*by Dr Naoto Ueno (Japan)*

High resolution imaging of malaria parasites: Light, X-rays or Electron? -*by Dr Leann Tilley (Australia)* [Chair: Dr Paul Matsudaira, Singapore]

Toward connectomics: making sense of the fly brain -*by Dr Ann-Shyn Chiang (Taiwan)*

Intracellular Ca^{2+} dynamics shapes the neural tube -*by Dr Dermot Kelleher (Singapore)*

Invited lectures and oral presentations:

Session I: Cell biology of viral infection [Chair: Dr Mah-Lee Ng and Dr Yasumasa Iwatani]

HIV-1 invalidates antivirl system of cellular APOBEC3 cytidine deaminases -*by Dr* Yasumasa Iwatani (Japan)

The role of host proteins in flavivirus replication -*by Dr Mah-Lee Ng (Singapore)*

MERS-CoV: receptor binding and fusion -*by Dr George F Gao (China)*

Cellular vimentin regulates construction of Dengue virus replication -*by Dr Justin Jang Hann Chu (Singapore)*

Synergistic interaction among begmovirus leads to suppression of host defense and resistance in chili plant -*by Dr Ashish K Singh (India)*

Session II: Animal stem cell biology [Chair: Dr K Vijay Raghavan and Dr Toshie Kai]

Stem cells to synapses: regulation of self-renewal and differentiation in the nervous system -*by Dr Andrea H Brand (UK)*

piRNA-biosynthesis and gene silencing -*by Dr Toru Nakano (Japan)*

Noncoding regulators of neural development -*by Dr Akshay Bhinge (Singapore)*

Hox gene function early in development to regulate hemangiogenesis and the neural plate border in zebrafish embryos -*by Dr Mark Featherstone (Singapore)*

Bantam miRNA: an essential candidate for brain development in *Drosophila* -*by Jagat K Roy (India)*

Session III: Cancer cell biology [Chair: Dr Kung Hsing-Jien and Dr Deng Lih Wen]

Change of cell identity: tumorigenesis and lineage coversion -*by Dr Lijian Hui (China)*

USP11 regulates PML stability to control Notch-induced malignancy in brain tumours -*by Dr Ruey-Hwa Chen (Taiwan)*

Real-time imaging of tumour dynamics and drug response in living tumours using FLIM-FRET microscopy -*by Dr Paul Timpson (Australia)*

Role of mixed lineage leukemia 5 in maintaining mitotic fidelity and genomic integrity -*by Dr Deng Lih Wen (Singapore)*

Formins drive migration of glioblastoma cells on linear patterns mimicking blood vessels -*by Dr Pascale Monzo (Singapore)*

Session IV: Parasite cell biology [Chair: Dr Theirry Diagana and Dr Celia R S Garcia]

Modulation of the *Plasmodium falciparum* transcription factor PfNF-YB by second messenger -*by Dr Celia R S Garcia (Brazil)*

Reverse chemical genetics of plasmodium -*by Dr Christophe Bodenreider (Singapore)*

Targeting ion transport in the malaria parsite with new generation anti-malarials -*by Dr Kiarn Kirk (Australia)*

Autophagy in *Trypanosoma brucei* requires acidocalcisomes -*by Dr Feng-Jun Li (Sinngapore)*

Biochemical characterization and chemical validation of *Trypanosoma brucei* Vps34 and Tbb PI4KIIIβ, potential molecular targets -*by Ujjini Manjunatha (Singapore)*

Session V: Autophagy and cell death [Chair: Dr Han-Ming Shen and Dr Tamotsu Yoshimori]

Caveolin-1 and lipid rafts in modulation of autophagy and lysosomal function -*by Dr Han Ming Shen (Singapore)*

New insights in membrane dynamics in autophagy -*by Dr Tamotsu Yoshimori (Japan)*

Autophagic lysosome reformation -*by Dr Li Yu (China)*

Biomarkers for dissecting necrotic cell death -*by Dr Liming Sun (China)*

Selective autophay in neurodegenertion -*by Dr Esther Wong (Singapore)*

Symposium VI: Organelle biology [Chair: Dr Mike Ryan and Dr Greg Jedd]

Structural and functional analysis of MiD51, a dynamij receptor required for mitochondrial fission -*by Dr Mike Ryan (Australia)*

Cell-free reconstitution of peroxisome tail-anchored protein biogenesis -*by Dr Greg Jedd (Singapore)*

Plasma membrane tethering of the cortical ER necessitates its finely reticulated architecture -*by Dr Zhang Dan (Singapore)*

Activation mechanism of the unfolded protein response during lipid disequilibrium -*by Dr Haoxi Wu (Singapore)*

A novel Ran-GTPase regulator required for flagellum functions -*by Dr Anais Brasseur(Singapore)*

Symposium VII: Cytoskeleton and cell cycle [Chair: Dr Mohan Balasubramanian and Dr Uttam Surana]

Mitotic cell division in plant cells -*by Dr Gohta Goshima (Japan)*

In vitro reactivation of contractile ring in fission yeast cell ghost -*by Dr Issei Mabuchi (Japan)*

RapGEF1, an inducer of differentiation, signals to the cytoskeleton and regulates catenin funcion -*by Dr Radha Vegesna (India)*

Integrin-matrix clustes from podosomes in the absence of traction forces -*by Dr Cheng-Han Yu (Singapore)*

Cortical regulation of cell size by a sizer cdr2p -*by Timothy Saunders (Singapore)*

Symposium VIII: Membrane trafficking [Chair: Dr Satyajit Mayor and Dr Wanjin Hong]

How vesicles find their targets at the Golgi -*by Dr Suzanne R Pfeffer (USA)*

A calcium dependent mechanism of secretory cargo sorting at the Golgi complex -*by Dr Vivek Malhotra (Spain)*

Parkinson's disease causing mutation alters retromer's function -*by Dr Rohan Teasdale (Australia)*

Stimulus-induced Munc13-4 recruitment releases granuphilin to prime fusion-reluctant docked granules -*by Tetsuro Izumi (Japan)*

Towards a molecular and ultrastructural understanding of caveolar morphogenesis -*by Dr Alexander Ludwig (Singapore)*

Symposium IX: Omics [Chair: Dr Bing Lim and Dr Zbynck Bozdech]

Systems biology of adipocyte dynamics and its regulation of metabolic homeostasis -*by Dr Rong Zeng (China)*

TBA -*by Dr Paul Robson (Singapore)*

Applications of metabolomics to understand the energetic basis of disease -*by Dr Scott Summers (Singapore)*

Probing metabolic regulation of cellular epigenetics using metabolomics -*by Dr Ng Shyh Chang (Singapore)*

High dimensional analysis of human T cell diversity with mass cytometry -*by Dr Evan W Newell (Singapore)*

Symposium X: Bacterial pathogenesis [Chair: Dr Linda Kenney and Dr Soman Abraham]

Cell death signaling during *E coli* infection -*by Dr Elizabeth L Hartland* (Australia)

Acidification of the *Salmonella* cytoplasm in the macrophage vacuole requires OmpR and drives type three secretion -*by Dr Linda K Kenney (Singapore)*

Biochemical dissetion of bacterial virulence and macrophage innate immunity -*by Dr Feng Shao (China)*

Colonization of lymph nodes by Yersinia pestis -*by Dr Ashley St John (Singapore)*

Symposium XI: Cell signaling [Chair: Dr Low Boon-Chuan and Dr Liou Yih-Cherng]

Identification of miRNA-491-5p and GIT1 as the modulators and biomarkers for a squamous cell carcinoma -*by Dr Lu-Hai Wang (Taiwan)*

Sculpting the mature nervous system: mechanisms of neuronal pruning in *Drosophila* -*by Dr Yu Fengwei*

Plasticity of cell signaling through a regulatory scaffold BCH domain -*by Dr Low Boon-Chuan (Singapore)*

Mechanical feedbacks regulating epithelial wound closure -*by Andrea Ravasio (Singapore)*

The physiological role of polycomb group protein Ezh2 in Langerhans cell -*by Jia Tong Loh (Singapore)*

Symposium XII: Cell biology education [Chair: Dr Cynthia Jensen and Dr Filipinas Natividad]

Teaching student excellence in scientific writing -*by Dr Kurt Albertine (USA)*

Usinng online resources to teach cell biology -*by Dr Omar Harb (USA)*

Teaching of protozoan parasites: Philippine setting -*by Dr Windell Rivera (Philippines)*

Membrane bound catechol-O-methyltransferase Val158Met A/G SNP and academic performance -*by Dr Josefino Castillo (Philippines)*

Symposium XIII: Growth and polarity [Chair: Dr Robert Arkowitz and Dr Yue Wang]

Phosphatidylinositol phosphate gradients during fungal filamentous growth -*by Dr Robert Arkowitz (France)*

The tumour suppressor ArgBP2 selectively stabilizes actin stress fibres and inhibits cell migration -*by Dr Edward Manser (Singapore)*

Partitioning zygote: establishment of head-to-tail axis in *C elegans* -*by Dr Fumio Mortegi (Singapore)*

Role of Arf G-proteins in the human fungal pathogen Candida albicans -*by Dr Martine Bassilana (France)*

Oscillatory dynamics of actin in a fission yeast mutant -*by Dr Dhivya Subramanian (Singapore)*

Symposium XIV: Bioimaging- Electron microscopy [Chair: Dr Yifan Cheng and Dr Shee-Mei Lok]

Structure determination of integral membrane protein by single particle cryo-EM -*by Dr Yifan Cheng (USA)*

Subnanometer resolution CryoEM structures of potent antibody complexed with dengue virus -*by Dr Shee-Mei Lok (Singapore)*

Visualization of the tiny part in a large macromolecular complex by cryo-EM imaging -*by Dr Wei-Hau Chang (Taiwan)*

Beyond the dot: correlative light and electron microscopy imaging of telomeres -*by Dr Sara Sandin (Singapore)*

Electron tomography of picoplankton: from cells to molecules -*by Dr Lu Gan (Singapore)*

Symposium XV: Evolutionary cell biology [Chair: Dr Peter Preiser and Dr Cynthia He]

The evolution of structural colour in butterfly wing scales -*by Dr Antonia Monteiro (Singapore)*

Evolution of cell shape changes and cell migration in algal morphogenesis -*by Dr Ichiro Nishii (Singapore)*

Growth as a mixed species biofilm consortia improves species fitness and reduces self generated diversity -*by Dr Scott Rice (Singapore)*

Evolutionary adaptation of mitochondrial dynamics and function in pathogen-host interaction -*by Dr Yunlong He (Singapore)*

Symposium XVI: Neurobiology [Chair: Dr Jongkyeong Chung and Dr Lim Kah Leong]

Parkinson's disease and mitochondrial dysfunction -*by Dr Jongkyeong Chung (Korea)*

Drosophila neural stem cells asymmetric division and tumour formation -*by Dr Hongyan Wang (Singapore)*

RNA editing and alternative splicing of $Ca_v1.3$ channels modulate neuronal excitability -*by Dr Yuk Wah Soong (Singapore)*

Mood improvement by deep brain stimulation: targets and mechanisms -*by Dr Lee Wei Lim (Singapore)*

A role for sorting Nexin 27 in AMPA receptors trafficking -*by Dr Li Shen Loo (Singapore)*

Symposium XVII: Bioimaging-Light microscopy [Chair: Dr Leann Tilley and Dr Li Hoi Yeung]

Super-resolution imaging of malaria parasites: sex and virulence -*by Dr Leann Tiley (Australia)*

A novel imaging method for systematic super-localiation of Golgi proteins -*by Dr Lu Lei (Singapore)*

Imaging mitosis: understanding cell fates following antimitotic drug challenge -*by Dr Randy Y C Poon (China)*

The zebrafish intestinal stem cells regenerate the epithelial layer along a base-to-tip axis -*by Dr Sahar Tavakoli (Singapore)*

Probing the nanoscale organization of the physical link between the contractile actin bundle and cadherin adhesion by superresolution microscopy -*by Dr Cristina Bertocchi (Singapore)*

Symposium XVIII: Microbial social behaviour [Chair: Dr E Peter Greenberg and Dr Lian-Hui Zhang]

Quorum sensing in Pseudomonas aeruginosa and a molecular mechanism for stabilizing cooperation -*by Dr E Peter Greenberg (USA)*

Quorum sensing and integrated quorum sensing systems -*by Dr Lian-Hui Zhang (Singapore)*

Regulation of the Type VI secretion system in *Burkholderia pseudomallei* through the sensing of thiols -*by Dr Yunn-Hwen Gan (Singapore)*

Community mediated interactions promote biofilm growth in iron limited environments -*by Dr Kimberly Kline (Singapore)*

Symposium XIX: Fungal pathogenesis [Chair: Dr Alex Andrianopoulos and Dr Naweed Naqvi]

Remodelling growth and survival in response to the host by *Penicillium* -*by Dr Alex Andrianopoulos (Australia)*

Surface sensing and signaling networks underlying morphogenetic transition during magnaporthe pathogenesis -*by Dr Naweed Naqvi (Singapore)*

Novel functions in pathogenesis of efflux genes, CDR1 and CDR2, in an azole-resistant clinical *Candida albicans* isolate -*by Dr Hsiu-June Lo (Taiwan)*

A role of bacterial-fungal interaction in *Candida albicans* infection -*by Dr Wang Yue (Singapore)*

Symposium XX: Cell biology of metbolism [Chair: Dr Peng Li and Dr David L Silver]

FIT2 deficiency in mice results in progressive lipodystrophy and metabolic dysfunction -*by Dr david L Silver (Singapore)*

FGF21 resistance in adipose tissues as a mediator of systemic insulin resistance -*by Dr Aimin Xu (China)*

Cellular responses to lipid perturbation -*by Dr Guillaume Thibault (Singapore)*

The ups and downs in AhpF: Structure, mechanism and ensemble formation of the alkylhydroperoxide reductase subunits AhpC and AhpF -*by Dr Gerhard Grueber (Singapore)*

Symposium XXI: Plant stem cells and development [Chair: Dr Toshiro Ito and Dr Hao Yu]

Epigenetic timing mechanism in Arabidopsis floral stem cells -*by Dr Toshiro Ito (Singapore)*

Molecular control of rice spikelet organ identity and meristem activities -*by Dr Dabing Zhang (China)*

Control mechanisms of meristematic activities in leaf primordia -*by Dr Hirokazu Tsukaya (Japan)*

FTIP3 and FTIP4 coordinately control shoot meristem development through regulting intracellular movement of STM -*by Dr Lu Liu (Singapore)*

The Executive committee of APOCB in its meeting elected Dr S Mayor, NCBS as the President of APOCB, Dr V Radha, CCMB as the Organizing Secretary of the 8th APOCB Congress, and decided to host the next meeting in the year 2018 in India.

Report prepared by
J K Roy
Cytogenetics Laboratory, Department of Zoology
Banaras Hindu University, Varanasi 221 005

Audited statement of accounts 2013-2014

MOHIT K. SAIGAL & CO.
CHARTERED ACCOUNTANTS

"SAIGAL HOUSE"
B 37/122, MAHMOORGANJ
VARANASI - 221010

INDIAN SOCIETY OF CELL BIOLOGY

BALANCE SHEET AS ON 31 MARCH,2014

LIABILITIES	AMOUNT	AMOUNT	ASSETS	AMOUNT	AMOUNT
CAPITAL FUND ACCOUNT:			INVESTMENTS		
Opening Balance	2,067,582.05		As per Annexure 'A'		2,409,962.17
Add: Excess of Income over Expenditure	81,660.00	2,149,242.05	CURRENT ASSETS & LOANS & ADVANCES :		
LIFE MEMBERSHIP FEES:					
Opening Balance	531,993.00		CASH & BANK BALANCES:		
			Cash	64.10	
			SBI, Varanasi	300,839.78	
Add: during the year	78,470.00	610,463.00	State Bank Of Hydrabad	48839.00	
			SBI IITR Branch	-	349,742.88
Audit Fees Payable					
TOTAL		2,759,705.05	TOTAL		2,759,705.05

For INDIAN SOCIETY OF CELL BIOLOGY

Executive Secretary
INDIAN SOCIETY OF CELL BIOLOGY

PLACE : VARANASI
DATE :

As Per Audit Report of Even Date

For MOHIT K SAIGAL & CO.
(Chartered Accountants)

MOHIT K. SAIGAL & Co. VARANASI CHARTERED ACCOUNTANTS

Mohit K. Saigal
FCA

MOHIT K. SAIGAL & CO.
CHARTERED ACCOUNTANTS

"SAIGAL HOUSE"
B 37/122, MAHMOORGANJ
VARANASI - 221010

INDIAN SOCIETY OF CELL BIOLOGY

Receipts & Payment A/c for the period 01.04.2013 to 31.03.2014

RECEIPTS		AMOUNT	PAYMENT		AMOUNT
To Opening Balances:			By Investment in FDR (HDFC)		350,000.00
Cash in Hand	318.10		By Printing & Stationery		1,185.00
SBI, Varanasi	288,525.78		By Workshop Exps		75,000.00
BOB, Varanasi	-		By Postage Exps.		78.00
SBI IITR Branch	55,769.00	344,612.88	By Bank Charges		3,537.00
To Membership Fees :			By Prof. J. Das lecture Exps.		33,118.00
Student & Ordinary	12,450.00		By Website mentanance Exps.		5,186.00
Life Membership Fees	78,470.00	90,920.00	By Student Award Exps.		5,000.00
			By Travelling Exps.		10,960.00
			By Audit Fees		5,000.00
To Interest on GOI			By Closing Balances:		
To Interest on SB A/c		8,141.00	Cash	64.10	
To Interest From HDFC		195,133.00	SBI, Varanasi	300,839.78	
To GOI Bond Matured			SBI IITR Branch	-	
To HDFC FDR Matured		200,000.00	State Bank Of Hydrabad	48,839.00	349,742.88
Total		838,806.88	Total		838,806.88

For INDIAN SOCIETY OF CELL BIOLOGY

Executive Secretary
INDIAN SOCIETY OF CELL BIOLOGY

PLACE : VARANASI
DATE :

As Per Audit Report of Even Date

For MOHIT K SAIGAL & CO.

MOHIT K. SAIGAL & Co. VARANASI CHARTERED ACCOUNTANTS

Mohit K. Saigal
FCA

INDIAN SOCIETY OF CELL BIOLOGY