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Dear Chairman,
I am responding to the advertisement posted on SCIENCE careers web-site, for the position of a tenure-track assistant professor in the Department of Biology, Indiana University, Bloomington IN. I am a senior research fellow working with Prof. Elliot M. Meyerowitz in the Department of Biology, California Institute of Technology, Pasadena.

I am studying the regulation of stem-cell niches in the shoot apical meristems (SAMs) of *Arabidopsis thaliana* by utilizing live-cell imaging, transient gene manipulation and genomic approaches. Stem-cell niches, in plants, represent a dynamic network of cells and they are maintained through a dynamic balance between self-renewal and differentiation. The regulation of stem-cell homeostasis, by cell-cell communication, can be best understood by employing systems based approaches. This includes resolving genetic networks that operate in stem-cell maintenance, through transient intervention and followed by real-time imaging of events that bring about niche re-organization. I have developed methods to transiently alter gene activities in wild type, living SAMs, and to record real-time changes in cell identities and cell behavior. I have utilized these methods to gain mechanistic insights into the regulation of stem-cell niche and SAM growth. This analysis has led to a conceptual framework and a tool-kit of reagents to explore, further, as to how multiple signals are integrated in space and in time to specify stem-cell fate, and to mediate orderly cell cycle transitions to maintain a functional stem-cell niche. I will continue to pursue studies aimed at resolving genetic networks regulating the stem-cell niche, which is as much a frontier in animal developmental Biology as it is in plant Biology. A real-time analysis of influence of genetic networks on determinants of growth and cell fate specification might not only reveal common themes regulating the diverse stem-cell niches, but could also lead to new concepts in developmental Biology.

I believe my research interests would complement the diverse areas of research being pursued at your department and has the potential to initiate collaborations with other groups. I am enclosing the copies of my curriculum vitae, research proposal and teaching interests for your consideration. I have requested the potential referees (listed in "BIOGRAPHICAL SKETCH" section) to forward their letters directly to your office.

Thank you
Yours sincerely

(G. Venugopala Reddy)

Research accomplishments and goals

Development of a multi-cellular organism from a single celled zygote is a fascinating and at the same time a complex biological problem. Cells divide, acquire distinct fates and differentiate into organs of definite sizes and shapes and with a specialized function. The challenge is to understand how cell-cell communication process are co-ordinated in space and in time to guide determinants of growth and differentiation. A combination of approaches including genetics, transient genetics coupled with real-time imaging and computational methods might lead to a better understanding of the process.

My research has revolved around gaining insights into the origin of cellular diversity and pattern formation by employing molecular genetics and live cell-imaging approaches in *Drosophila melanogaster* and *Arabidopsis thaliana*. My graduate work was aimed at understanding cell fate specification in the *Drosophila* antenna, which harbors regularly spaced olfactory neurons. My work has shown that olfactory system utilizes both recruitment and lineage based mechanisms to achieve cell diversity required to make a sensory unit (1). This study has formed the basis for subsequent work aimed at understanding the functional and spatial organization of odor perception and pattern formation in the olfactory lobe, a higher center in the brain. The most significant contribution, however, is the work which led to the revision of mechanosensory lineage. My work shows that an additional division within the mechanosensory lineage results in additional cell, which acquires glial cell identity and migrates away from the sensory cluster (2, 3). This work has contributed to several new studies aimed at re-examining the cell-cell communication and origin of cellular diversity in the peripheral nervous system. My graduate work provided me with an introduction and insights into principles and mechanisms of development and approaches to address intricacies involved in the process.

My decision, to switch, to study development of shoot apical meristems (SAMs) is guided by my long-standing interest in tissue homeostasis. Cells in developing tissues maintain a life-long homeostatic balance in which growth and differentiation is dynamically regulated. The SAMs of flowering plants have a remarkable ability to produce new organs continuously, in some cases for thousands of years, and yet maintain a relatively constant pool of stem cells. The common animal mechanisms such as cell migration and programmed cell death are not utilized in SAM development. Therefore, the growth in SAMs has to be regulated primarily at the level of cell division and cell expansion patterns, minimizing the complexity of analysis. The functional domains of cells in SAMs are established in embryonic development and maintain their identity and function, throughout post-embryonic life, in a dynamic environment. The regulation of their function requires an understanding of how cell-cell signaling mechanisms impinge on determinants of growth and differentiation, in real-time. I have developed new methods to transiently alter cell-cell communication and to record SAM re-organization and cell behavior, in real-time (4). This analysis has not only yielded novel insights, but also provides a conceptual framework to guide future studies on stem-cell maintenance (5). A high-resolution data, obtained from real-time observations, should aid in developing a computational platform to model and test regulatory interactions in developing SAMs (6). My future research revolves around utilizing dynamic imaging approaches to understand how multiple signals are co-ordinated in space and time to guide cell fate decisions and cell cycle transitions to maintain a functional stem-cell niche.

References

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5. **G. V. Reddy** and E. M. Meyerowitz *Science* (*Science express*, 6 October 2005)
6. **H. Jonsson** *et al.*, *Bioinformatics* **1;21** Suppl 1:i232-i24

Teaching experience and interest

I have developed a strong interest in teaching over the years. My inclination towards teaching was influenced by my own real-life experiences during primary school education. It is a memorable experience to recollect that some teachers made learning simpler, fun and effective. Though I could not define teaching qualities at that time, but my own experiences in later years have taught me a few lessons about effective teaching.

I was introduced to teaching during my Master's degree program. I contributed as a teaching assistant for Crop Physiology course designed for undergraduate students. It was an introductory course dealing with the basic aspects of structure and function of plant cells and tissues, mineral nutrition and metabolism. My responsibilities included providing help in preparing lecture notes, presentation slides and evaluating assignments for the lecture series. For the practical sessions, I was responsible for introducing the basic principles of experiments, planning the experiments, preparing the laboratory with necessary tools and reagents and evaluating the individual assignments. Later during my Ph.D. program, I volunteered to teach in a course entitled "Developmental Genetics" designed for Master's students and the students from "visiting Summer Research Program (VSRP)". This is a broadly structured course covering the basic aspects of developmental mechanisms both in plants and in animals, with a special emphasis on genetic model systems. As a part of this course, I made lecture presentations on topics related to the mechanisms of origin of cellular diversity and pattern formation in multi-cellular development. More specifically, I covered topics related to the roles and regulation of extrinsic signaling mediated by cell surface receptors such as Notch and Epidermal Growth Factor Receptors (EGFRs) and the intrinsic bias created by asymmetric segregation of factors such as NUMB and PROPSERO. The teaching opportunities during post-doctoral studies were minimal and mostly centered around mentoring undergraduate students on their Summer research projects.

Personal reflections and the feed back received from students have been useful in refining my teaching philosophy and skills. Apart from the basic skills such as firm knowledge over subject matter and effective communication, several other factors are crucial for effective teaching. The major aspect of effective teaching is to make the class interesting and relevant by relating the subject matter to a closest thing that can be easily understood. Making the class interactive, giving importance to the thoughts of students and encouraging independent thinking can make the class interesting. Finally, the respect and the concern for students are the motivating factors which can bring the best in a teacher.

Teaching has been a learning and a rewarding experience for me in many ways. It has enriched me with the knowledge and helped me to clarify my thoughts on several concepts. Therefore, I maintain a strong interest in teaching.

Resolving genetic networks regulating the stem-cell niche: Transient genetics and dynamic imaging

Stem-cells are maintained in interactive micro-environments, termed as niches. Properties of self-renewal and pluripotentcy, makes stem-cell niches a system of choice to explore the mechanisms governing the balance between self-renewal and differentiation. Understanding how multiple signals are co-ordinated in space and in time to influence patterns of gene expression, and cellular behavior is critical to understanding stem-cell homeostasis. Such an understanding requires a systems level analysis involving transient perturbations in gene regulatory networks and real-time imaging of the niche re-organization. A dynamic analysis might reveal common properties regulating the stem-cell niches, which are maintained in diverse tissue types, both in animals and in plants (1).

In plants, the stem-cell niche resides at the tip of each shoot, the shoot apical meristem (SAM) and provides the cells that will differentiate to leaves, flowers, and stem. In *Arabidopsis thaliana* the SAM consists of several hundred cells (5μM each) divided into functional domains that are characterized by different cellular behaviors, and by different patterns of gene expression (2) (Fig. 1). The central zone (CZ) is at the tip and harbors a set of stem-cells (Fig. 1A, E). Progeny of stem-cells enter into differentiation pathways when they enter the surrounding regions – the flanking peripheral zone (PZ; Fig. 1B, F) where leaf and flower primordia are formed (Fig. 1D), and the rib-meristem (RM) beneath the CZ where cells of the stem form (Fig. 1C, G). The domains are established in embryonic development, and maintain their relative proportions throughout postembryonic life, despite cells being continually diverted to differentiation pathways. SAM, thus, represents an interacting network of functionally distinct domains, their identity and function are dynamically regulated, by cell-cell communication and hence requires a dynamic analysis to understand the process.

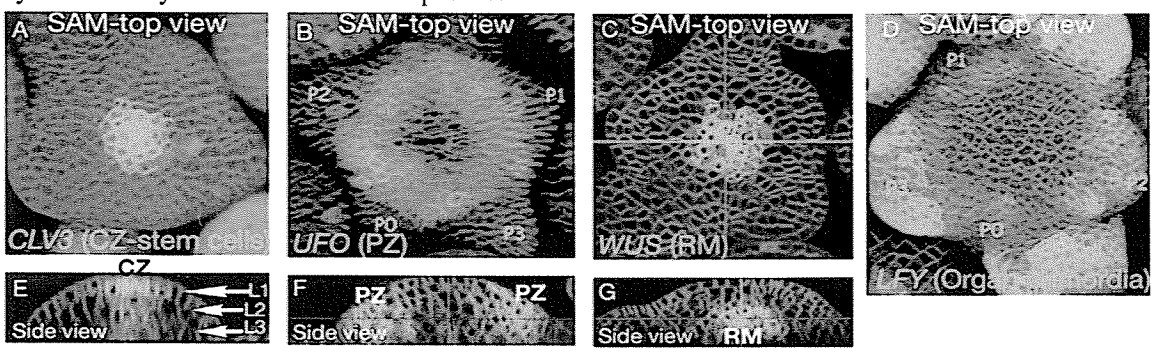

Fig. 1: Functional sub-domains within the SAM, marked by promoter::*mGFP5-ER* reporter constructs. (A), (B) and (D) are the 3-D re-constructed SAMs, highlighting the stem-cell domain marked by *CLAVATA3* promoter (A), the adjacent PZ is marked by *UNUSUAL FLORAL ORGANS (UFO)* (B), RM revealing *WUSCHEL* expression (C), and the sites of differentiation are marked by *LEAFY* (D). (E-G) are the re-constructed side views of SAMs represented in (A-C) respectively, revealing 3 clonal layers of cells. Cell outlines are highlighted by FM4-64 dye (red).

Multiple signaling networks have been implicated in stem-cell homeostasis. **a) CLV-WUS feed-back loop:** The stem-cells signal to the RM through CLAVATA3 (CLV3), a small extracellular protein thought to be the ligand for the CLAVATA1 (CLV1) receptor kinase, expressed in RM cells (3, 4). CLV1 acts by downregulating the activity of the homeodomain protein WUSCHEL (WUS), also expressed in RM cells (5, 6). WUS acts, in turn, to upregulate *CLV3* expression in the overlying stem-cells, via an unknown diffusible signal (7). CLV3 thus regulates its own expression and hence the stem-cell niche, through a feedback network involving CLV3-expressing stem-cells in the CZ and the cells of the RM (8) (Fig. 2A, B). **b) The function of SHOOT MERISTEMLESS (STM)**, a homeodomain transcription factor, adds another layer

of regulation in SAM establishment and/or maintenance (8) (Fig. 2C). *stm* mutants fail to develop a functional SAM and STM has been proposed to function in maintenance of cell proliferation by repressing genes such as *ASYMMETRIC LEAVES1 (AS1)*, which are expressed in differentiating cells within the PZ (9). **c) Plant hormone auxin** has been shown to mediate the choice of differentiation sites within the PZ (10) (Fig. 2D). These studies infer a role for short range signaling between adjacent groups of cells in regulating stem-cell homeostasis and SAM growth.

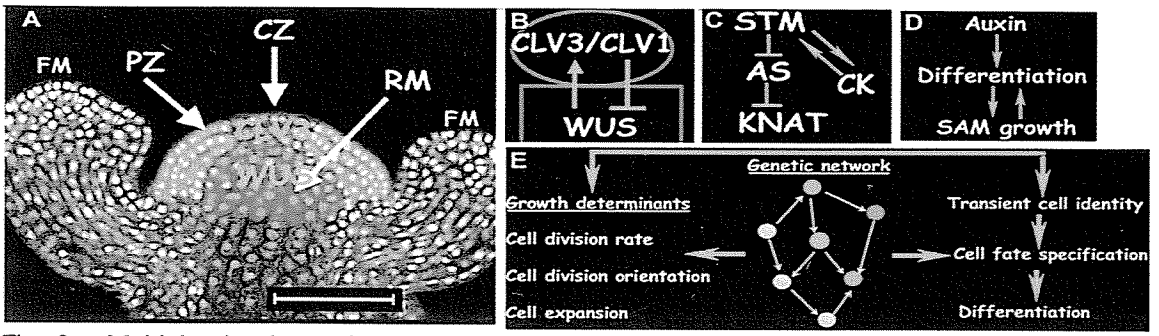

Fig. 2: Multiple signals are implicated in stem-cell homeostasis. (A) side view of SAM depicting functional domains, the domains. (B-D) schematic representations of gene networks, implicated in stem-cell homeostasis and SAM growth. (E) Conceptual representation of a genetic network, influencing determinants of growth and patterning. *KNAT>>>KNOTTED1-LIKE, CK>>>cytokinin.*

However, ordinary analysis of the mutants thus gives clues to the network of interactions, but is not adequate to define the function of individual genes in the network of interacting cells. This is because loss or gain of function of one component affects the entire network, so that the ultimate mutant phenotype, assessed long after the initial effects of the mutation, is the result of a long series of events that affects the expression of many genes. Understanding the function of individual genes in an interacting network of cells, such as the SAM, would involve, **A) creating transient perturbations in gene activities by turning them off/on in a living, wild-type SAM, B) Following in real time the changes in the organization of the SAM, and in the rates and patterns of cell division.** By doing this we can not only see the immediate effect of loss/gain of gene activity on transient cell identities and cell behavior, but also the interplay between changing cell identities and cell behavior (Fig. 2E). Such an analysis might shed light on mechanisms governing stem-cell homeostasis and SAM growth. I have developed methods to transiently alter gene activities in wild type SAMs and to record real-time changes in cell identities and cell behavior. I have utilized these methods to study the process of stem-cell homeostasis. A brief description of my work, published thus far, is given below.

1 Real-time lineage analysis reveals oriented cell divisions associated with morphogenesis at the shoot apex (11) (Reddy *et al.*, *Development* 2004)

This study describes a novel live imaging technique to record cell division and cell expansion patterns in living SAMs (Reddy et al., 2004). The real-time analysis of cell behavior has led to an integrated map of spatio-temporal patterns of cell division orientation, rates of cell division and cell expansion in SAMs, and it can form the basis for future studies aimed at seeking a causal link between cell-cell communication, SAM organization and growth.

2 Stem-cell homeostasis and growth dynamics can be uncoupled in the *Arabidopsis* shoot apex (12) [Reddy and Meyerowitz, *Science*, (*Science express*, 6 October 2005)]

Several hypothesis have been proposed to explain the terminal phenotypes of *clv3* mutants which display a massively enlarged stem-cell domain and overall increase in SAM size (3, 4). In this study, I have combined dexamethasone (DEX) inducible, double-stranded RNA

interference (dsRNAi) mediated, silencing of *CLV3* and real-time imaging to demonstrate that the effects of CLV communication network on restricting stem-cell domain and SAM growth can be uncoupled (Fig. 3). This analysis has provided mechanistic insights into the function of CLV

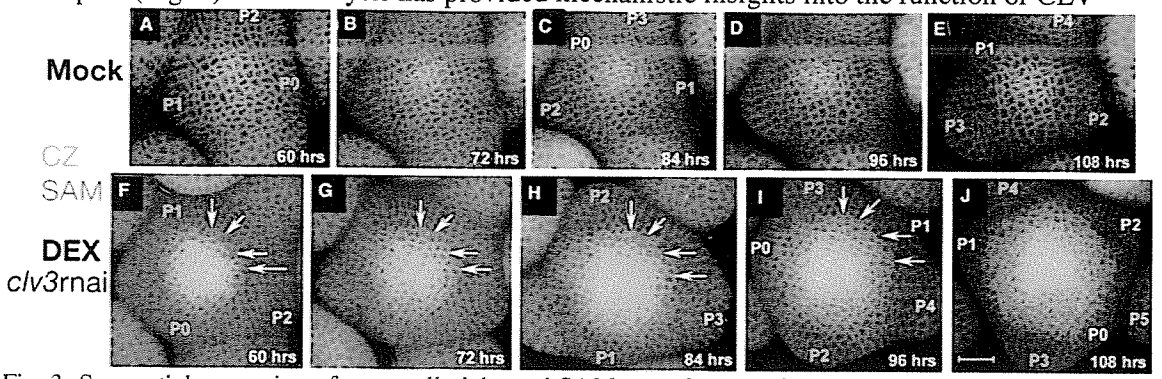

Fig. 3: Sequential expansion of stem-cell niche and SAM growth, upon CLV3 removal. (A-E) and (F-J) are the reconstructed views of the SAMs mock treated or treated with DEX, respectively. Time elapsed, post treatment, is marked. The CZ behavior (p*CLV3*::*mGFP5-ER*, green) is followed with a cell division marker (*35S*::*YFP29-1*, red). Scale bar, 20μM

signaling in regulating the stem-cell niche and the SAM size. It provides a conceptual framework to explore, further, the mechanisms by which cell-cell communication networks mediate stem-cell maintenance and growth control.

I propose to utilize my expertise in live-imaging, a tool-kit of reagents I have generated, to address the following questions,

1) **How multiple signals modulate CLV-WUS feed-back network (a central regulator of stem-cell niche) to mediate stem-cell homeostasis and SAM growth?**
2) **How multiple signals are co-ordinated in space and in time to mediate cell cycle transitions to maintain a functional SAM?**
3) **I will combine transient gene silencing, stem-cell enrichment strategy and expression profiling to identify components of CLV-WUS network.** A brief description of specific objectives and preliminary data, in each case, is accounted below.

1. Regulation of stem-cell niche and SAM growth

1.1 Uncoupling of stem-cell specification and SAM growth

1.1.1 Dedicated receptor kinases for mediating stem-cell specification and growth?

CLV3 functions both to restrict the stem-cell domain and the SAM growth. Transient removal of CLV3 activity and live imaging has revealed that the mechanisms which mediate stem-cell specification and growth could be different (Fig. 3). What are the mechanisms by which CLV signaling regulates stem-cell specification and SAM growth? Available evidence suggests that CLV3 might function as a ligand to activate not only CLV1 receptor, but also structurally related receptor kinases (13). Atleast three receptor kinases, which share sequence homology with CLV1 have been documented. Therefore, it is possible that separate receptor kinases function, downstream of CLV3, to mediate stem-cell specification and growth. The following experiments would test such a possibility. **a) Transient inactivation of CLV1 and CLV1 family members followed by real-time imaging of SAM organization and growth.** The inactivation of CLV1 receptor is expected to enhance CLV3 levels through a regulatory feedback loop which, in-turn, might activate structurally related receptor kinases and thus establishing an equilibrium condition. Such an equilibrium would not reveal the true function of receptor kinases, when analyzed by using terminal phenotypes. Therefore, it is essential to analyze the function of each receptor kinase by generating transient perturbations, in real-time experiments. **b) Transient over-**

expression of CLV3 in *clv1* mutants and in mutant backgrounds of structurally related kinases, and followed by real-time imaging of SAM organization and growth. This analysis would reveal the relative contribution of each receptor kinase in mediating stem-cell specification and SAM growth and thus providing clues to the mechanisms by which stem-cell specification and growth can be uncoupled.

1.1.2 Exploring the interface between CLV-receptor kinase signaling and growth control networks

CLV signaling, which functions within the CZ, mediates a long-range inhibition of cell division activity within the PZ (12). What are the mechanisms which mediate such a long range effect on growth? Studying the interaction between CLV-WUS signaling network and growth control networks, in real-time experiments, could provide clues. Gene regulatory network controlled by STM, has been implicated in SAM establishment and growth (Fig. 2C). Since *stm* mutants fail to make embryonic SAM, cellular function of STM has not been understood. A failure to make SAM either indicates lack of availability of cells or defective patterning leading to growth arrest. In this context, it will be illuminating to test the interaction, in-real-time experiments, between CLV-WUS network and STM network. Specific experiments to test STM function and its interaction with CLV-WUS include,

a) Creating transient and inducible perturbations in STM activity and analyzing its effects on SAM organization (Fig. 1A-D), on key regulators of CLV-WUS cascade (Fig. 4A-C) and cell behavior. **b) Analysis of STM levels, in real-time experiments, upon compromise of CLV3 function**. It should be possible to extend this analysis to test the function of several other signaling networks implicated in maintaining a functional SAM. Experiments outlined in this section could provide a link between CLV receptor network and growth control elements.

1.2 CLV-WUS network: Regulation of stem-cell niche

1.2.1 Properties of CLV-WUS feed-back network and regulation of stem-cell niche

The proposed function of CLV signaling is to confine WUS to rib-meristem cells and WUS acts, in turn, to promote stem-cell fate in the overlying CZ cells (Fig. 5A). However, properties of CLV-WUS feeb-back network are poorly understood and hence seeking answers to the following questions is critical to understanding the regulation of stem-cell niche. Whether CLV signaling functions to restrict WUS levels or domain of expression? Whether WUS levels or spatial expression, is crucial in regulating the stem-cell niche? What is the range of CLV3 diffusion? What is the domain and pattern of CLV1 receptor activity? Where does WUS map with respect to CLV1 activity? The following experiments could reveal the properties of CLV-WUS feed-back loop. **a) Towards a CLV1-receptor activity map.** I propose to utilize fluorescence resonance energy transfer (FRET) based approach to visualize CLV3-CLV1 interaction, in vivo. A real-time analysis of FRET signals would facilitate the visualization of

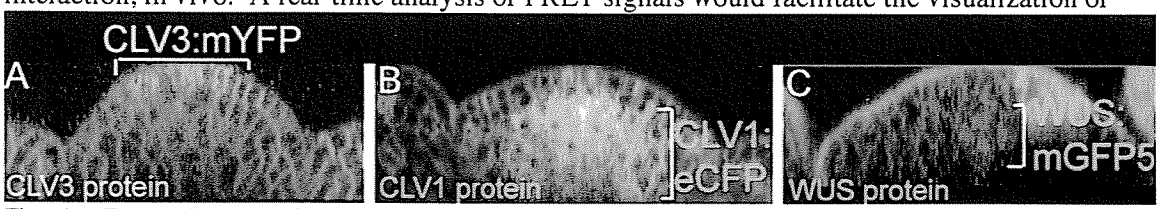

Fig. 4: Expression domains of functional fusion proteins of key regulators of CLV-WUS interaction network. (A), (B) and (C) are the side views of CLV3:mYFP, CLV1:eCFP and WUS:mGFP5 respectively, expressed from native promoters. Reddy, G.., unpublished results.

CLV1 receptor activity (CLV3-CLV1 binding is assumed to activate CLV1 receptor). The fusion proteins between spectrally variant fluorescence proteins and CLV3 (CLV3:mYFP), CLV1 (CLV1:eCFP) and WUS (WUS:mGFP5) have been generated (Fig. 4; Reddy, G. V., unpublished

results). **b) A real-time analysis of WUS protein levels (Fig. 4C) and the *WUS* expression domain (Fig. 5A)** both in wild type and upon transient removal of CLV3 function (Fig. 3) could provide insights into the nature of negative regulation imposed on WUS by CLV signaling. **c) Manipulation of spatial expression of WUS and levels,** followed by real-time monitoring of re-organization pattern of stem-cell niche could provide insights into the WUS mediated regulation of stem-cell niche.

1.2.2 Exploring novel modes of signaling: Cell ablations, transient gene manipulation and real-time imaging

The mechanisms by which WUS mediated signal communicates with the overlying cells in the CZ, to promote stem-cell fate are not known. We have observed a gradual radial expansion of the stem-cell domain, following the reduction in CLV3 activity (12) (Fig. 3F-J). Therefore, it indicates that WUS might activate a stem-cell promoting signal that spreads from the CZ with time. If the signal does spread with time, it could do so either by diffusion, or by stepwise

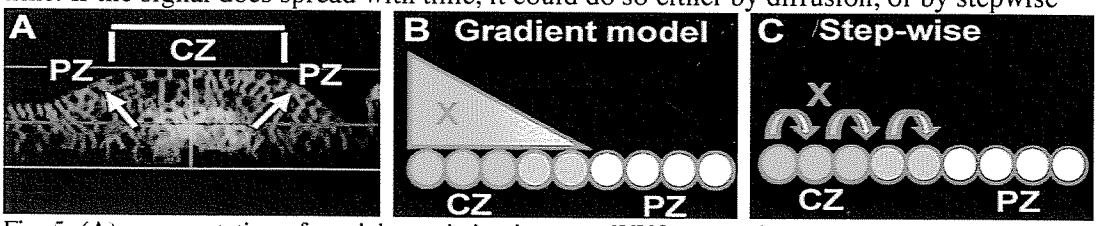

Fig. 5: (A) representation of spatial correlation between WUS expression domain and limits of the CZ. (B) and (C) represent possible models explaining, the properties of an hypothetical factor"X" involved in regulating the radial dimensions of CZ/stem-cell domain.

communication between adjoining cells (Fig. 5B, C). The following experiments could reveal the properties of such a signal. **a) Ablation of the CZ cells and followed by inactivation of CLVsignaling. b) Ablation of the CZ cells and followed by ectopic over expression of WUS.** Both the conditions can be expected to prevent the gradual expansion of stem-cell domain, if the signal spreads radially outward from the CZ. Thus, a combination of physical and transient gene manipulation, and live-imaging can provide clues to the novel modes of signaling regulating the stem-cell niche.

The experiments proposed in sections 1.1 and 1.2 should form the basis for a NSF grant proposal, with preliminary data and the tool-kit (Fig. 1, Fig. 3 and Fig. 4) available to carry out the proposed experiments.

2. Developmental regulation of cell cycle transitions: Order and specificity

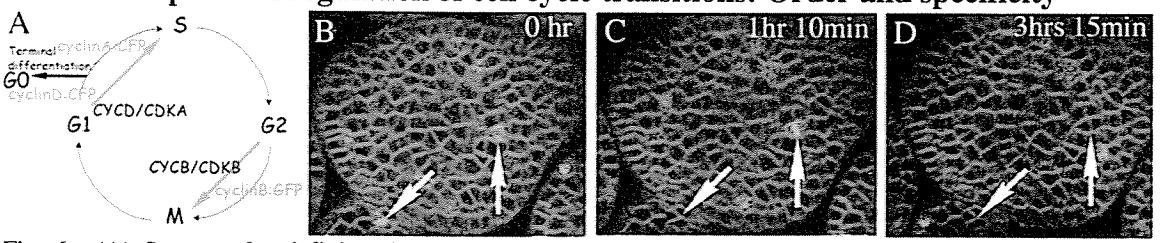

Fig. 6: (A) Strategy for defining the temporal parameters of cell cycle transitions in SAM cells. (B-D) sections of a SAM from a time-lapse series indicating cyclinB1;1:GFP dynamics (green). Elapsed time is indicated. Cell outlines highlighted with FM4-64 (Red). Arrows point to the same cells over time.

Stem-cell homeostasis and SAM growth, almost, entirely depend on spatial control of cell division and cell growth, as the mechanisms of programmed cell death and cell migration are not utilized (11, 12). Several regulatory networks (Fig. 2), plant hormones and environmental signals such as light and temperature have been implicated in SAM growth. Understanding how

cell-cell communication and cell growth networks are integrated in space and in time to mediate cell cycle transitions is central to understanding stem-cell homeostasis. A real-time analysis of the effects of transient manipulations of regulatory networks on cell cycle transitions should yield high resolution data to seek answers to the following questions.

A) **How do signals, which control cell size and developmental signals interact to guide cell cycle transitions?**

B) **How do external signals such as light, and developmental signals interact to mediate cell cycle transitions?**

I propose to employ dynamic imaging to determine the temporal parameters of cell cycle transitions for all cells in SAMs. This can be achieved by generating spectrally variant fluorescent protein markers to visualize G1>>>S and G2>>>M transitions, in the same cells, through dynamic imaging (Fig. 6A). The analysis should yield a dynamic and integrated map of cell cycle transitions for all SAM cells, revealing the temporal parameters of transitions. Such a template can then be used, as a reference, to investigate the temporal windows in which each one of the key developmental signals function, to regulate cell cycle progression. This can be achieved, by generating transient perturbations in regulatory networks and then analyzing their effects on temporal parameters of cell cycle transitions. Such an approach could yield insights into, specificity and order in which developmental signals function to mediate cell cycle transitions and guide future experiments aimed at identifying specific cell cycle components, targeted by developmental signals. Specific aims of this proposal are listed below.

a) To generate an integrated four-dimensional map of cell cycle transitions. In *Arabidopsis*, cell cycle machinery consists of approximately 30 cyclins, grouped into 3 classes (A-type, B-type and D-type) and two classes of cyclin dependent kinases (CDKA type and CDKB type) (14). My work has revealed CYCLINB1:GFP dynamics, in living SAMs, highlighting G2>>>M transition (Fig. 6B-D). The fluorescent markers to illuminate G1>>>S transition, in same cells, will be generated and this process involves identifying the right sets of CYCLIND that are expressed in the SAM cells and generating functional fluorescent protein tags, such as CYCLIND:CFP.

b) Analyzing the effects of transient perturbations in cell-cell communication networks on temporal parameters of cell cycle transitions.

c) Analyzing the effects of transient manipulations in determinants of cell growth on temporal parameters of cell cycle transitions.

I believe, this exercise will be a major step forward in establishing a link between signaling pathways mediating stem-cell maintenance and cell cycle machinery and may guide future studies, asking a broader sets of questions related to the control of cell size and cell division in developing fields. This area of research is as much a frontier in animals developmental fields as it is in plant kingdom (15). **Experiments described in this section, along with the descriptions of transient manipulation of key regulators of cell growth and cell division, should form the basis for a separate grant proposal.**

3. Signaling components

The components acting downstream of CLV1 receptor to repress WUS and mechanisms by which WUS signal reaches back to the CZ are elusive. I propose to employ both genomic and genetic approaches to isolate additional components of stem-cell maintenance network.

3.1 Inducible gene silencing and expression profiling: I have combined inducible *CLV3* gene silencing (Fig. 3) system and stem-cell enrichment provided by *apetala1;cauliflower* mutant (they make numerous SAMs) background, to profile genome wide changes in gene expression, upon compromise of CLV3 function. Analyzing their RNA expression patterns and functional characterization of relevant sub-sets of genes, through transient genetics methodology, might provide missing links in CLV-WUS communication network. This analysis should also yield cell type specific markers.

3.2 Tissue specific mis-expression screens: I have employed two component transcriptional activation system to drive expression of array of genes, through random insertional mutagenesis, into the CLV1 expression domain/rib-meristem and it has yielded several dominant phenotypes. Further characterization of these phenotypes might yield novel components of stem-cell regulatory network.

Transient intervention and dynamic imaging approaches would allow the dissection of the influence of genetic networks on stem-cell specification and SAM growth. Such an analysis provides high resolution data to model gene regulatory networks (GRNs), which in turn, should guide in-vivo experiments. Therefore, I maintain interest in collaborating with research groups and individuals, with research interests in developing computational methods to model GRNs in multicellular contexts (15) (www.computableplant.org).

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