13:35

Meristem formation in the *Arabidopsis* shoot and its modification

Mitsuhiro Aida (Developmental Morphology)

Plant shoot organs such as leaves, stems, and flowers, are all generated from the shoot meristem, a small stem-cell containing tissue located at the tip of the stem. Cells in the meristem proliferate to produce organ primordia from its periphery while they self-renew to maintain themselves. Typically, shoot meristems are formed multiple times at multiple locations during the plant lifecycle and properties of each meristem, including its activity, size, or types of organs it differentiates, are tightly controlled depending on developmental contexts and environmental conditions. Our group has focused on molecular mechanisms of meristem formation in three different developmental contexts in *Arabidopsis thaliana*: embryogenesis, flower formation, and female reproductive organ formation, and uncovered key regulatory processes in each context. In embryogenesis, the redundant transcription factors CUC1 and CUC2 promote expression of several regulatory genes that promote meristem activity while they also promote expression of a gene that restricts meristem size and organ production rate, showing that embryonic shoot meristem formation involves coordinated activation of two classes of genes with opposing functions on meristem activity. In flower and female reproductive organ development, we have uncovered interactions between general factors for meristem formation and those that modify each of the processes specific to their contexts. Thus, modification of general developmental programs is a key to understand the diversity in plant organ forms.

14:00

Implications of parental genome imbalance for plant endosperm development

Tetsu Kinoshita (Plant Reproductive Genetics)

Maternally and paternally inherited genomes do not contribute equally to their progeny in mammals or flowering plants. Rather, the two genomes act antagonistically in the embryo-nourishing tissues, the placenta and endosperm. In flowering plants, endosperm development is normal in crosses between plants of the same species, but often aborts in crosses between species or with different levels of ploidy. A long-standing hypothesis proposes that this type of parental genome imbalance is caused by a sub-set of genes that are expressed exclusively from the maternal or paternal genomes; however, little is known about the possible underlying molecular mechanisms. In this seminar, I describe evidence showing that Polycomb Repressive Complex 2 (PRC2), an evolutionarily conserved multi-protein complex able to methylate histone H3K27, has a crucial role in rice endosperm development in interspecific crosses. Hybrid endosperms show altered developmental transitions depending on the species used for the intercross. Additionally, gene expression patterns, the up- or down-regulation of *OsMADS87* (putative target of PRC2), and endosperm developmental transitions are correlated in each interspecific cross. We also found that a component of PRC2, *OsFIE1*, was exclusively expressed from the maternally inherited genome in the endosperm but not in the embryo in any intercross combination. I propose that this epigenetic pathway plays a central role for endosperm development, and also form a reproductive barrier that occasionally allows emergence of new species by interspecies hybridization among balanced genomes.

14:25

Evolution of a tissue-specific silencer underlies divergence in the expression of paralogues

Hajime Ogino (Developmental Genomics)

Recent analyses of teleost-specific paralogues suggest a role for the differential degeneration of duplicated enhancers in the evolutionary diversification of paralogue expression. However, no one has reported evidence for the involvement of innovative *cis*-regulatory changes. Here we show that silencer innovation diversified expression of the paralogues, *pax2* and *pax8*, in *Xenopus*. *pax2* shows multi-tissue expression, as does the ancestral amphioxus orthologue, *pax2/5/8*, whereas *pax8* expression localizes to a subset of *pax2*-expressing tissues. We revealed that, despite their diverged expression, both *pax2* and *pax8* retain ancestral modes of *cis*-activation. *pax8* is associated with multiple enhancers, each of which is capable of directing *pax2*-like, multi-tissue expression, and one of these *pax8* enhancers is conserved in *pax2*. However, a silencer within the *pax8* proximal promoter suppresses their pleiotropic enhancer activity outside the *pax8*-expressing tissues. In contrast, the combination of the *pax2* proximal promoter with either the *pax8* or *pax2* enhancer recapitulates *pax2*-like expression, as in the amphioxus *pax2/5/8* promoter. We propose that silencer innovation, rather than enhancer degeneration, was crucial for the divergent expression of paralogues with pleiotropic enhancers inherited from their common progenitor. Furthermore, silencer innovation might be an evolutionary escape route for genes whose expression is robustly regulated by redundant enhancers, such as *pax8*.

14:50 Break

15:00

Analysis of mechanisms to regulate stress signal transduction by a glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase

Susumu Morigasaki

Cells maintain intracellular conditions even whereas extracellular (environmental) conditions are always fluctuating. Severe environmental changes that threaten life or cause growth defect are referred to as “stress”. A major and important response is induction of stress “resistance” gene expression. The other crucial action is “optimization” that allow cells to restart growth under the new conditions. Adaptation is a result of cooperation of “resistance” and “optimization”.

My colleagues and I have been focusing on the systems of “sensing and signaling”, because these are the earliest steps of stress response. My research effort is on two signaling pathways involving “stress-activated protein kinase (SAPK)” and “target of rapamycin (TOR)”. The former mainly plays a pivotal role for stress “resistance”. The latter is involved in “optimization” through control of the fundamental cell functions (e.g. cell cycle and metabolism).

In the fission yeast *Schizosaccharomyces pombe*, disruption of the *tdh1*$^{+}$ gene encoding a glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) significantly decreases H_2O_2-induced phosphorylation of SAPK, Spc1 and a substrate of TOR, Gad8 that belongs to the AGC kinase family. Furthermore, Tdh1 physically associates with the upstream components of both kinases. Intriguingly, the Tdh1-targets form complexes; i.e. a MAP three kinase complex (MTKC) and a TOR complex2 (TORC2), respectively.

Therefore, we proposed that Tdh1 controls adaptation through coordinating both signaling pathways for “resistance” and “optimization”. To make this idea more convincing, I have investigated (1) characters of MTKC and TORC2; (2) mechanisms to regulate these complexes; and (3) function of Tdh1 on the stress signal transduction.

15:20

Developing a genetic assay for the analysis of protein disulfide bond formation in the ER of mammalian cells

Hiroshi Kadokura

Disulfide bond formation is essential for the folding of cell surface proteins. Despite the discoveries of numerous factors potentially involved in the process, their roles in living cells remain largely unknown (1-4). To reveal their functions in mammals, I took two approaches. First, to elucidate the roles of disulfide enzymes, I devised a method that enables us to identify the substrates of each enzyme from mouse tissues (5). Second, I engineered firefly luciferase to report defects in disulfide bond formation in the ER. The reporter finally constructed is highly sensitive and makes it possible to study the roles of each factor under stress conditions (e.g. low oxygen), or to screen small molecules that affect the process.

Maintaining the quality of cell surface proteins is vital for organisms. I directly supervised students (6-8) or collaborated with researchers (9-10) to discover sophisticated mechanisms involved in the process (6-10).

Publications:
1. 門倉　広　化学と生物　48, 695-705　(2010).
2. 門倉　広 Protein Community 6, 35-36 (2010).
3. Kadokura and Beckwith. Cell 138, 1164-1173 (2009).
4. Kadokura and Beckwith. Antioxid. Redox Signal. 13, 1231-1246 (2010).
5. Kadokura et al. (in preparation).
6. Shinya, Kadokura, et al. Nucleic Acids Res. 39,5245-5254 (2011).
7. Yamamoto et al. Cell Struct. Funct. 35, 107-116 (2010).
8. Sopha, Kadokura et al. (in preparation).
9. Yanagitani et al. Science 331, 586-589 (2011).
10. Ohtsu et al. J. Biol. Chem. 285, 17479-17487 (2010).

15:40

The elucidation of plant-specific DNA damage checkpoint

Kaoru Yoshiyama

DNA can be damaged by extracellular and intercellular insults such as ionizing radiation, chemical agents and reactive oxygen species.　Therefore, eukaryotic cells have DNA checkpoint systems, which arrest the cell cycle to allow time for the cell to repair damaged chromosome before entering mitosis.

In mammals, ATM (Ataxia Telangiectasia Mutated) and ATR (ATM and Rad3-related) kinases sense DNA stress and transmit the signals to the downstream proteins.　p53, a tumor suppressor protein, is one of targets of these kinases, and regulates transcription of target genes involved in the DNA damage response.

Plants' life style, which is sessile and photosynthetic, is completely different from mammals'. Therefore, we expect that plants are continuously exposed to DNA damage stress from environments than mammals.　Although plants also have ATM and ATR homologs, p53 homologs and some of other DNA checkpoint proteins identified in mammals are missing in plants.

We previously showed that *Arabidopsis* SOG1 (suppressor of gamma response) is a novel transcription factor that governs DNA damage response, and observed the functional parallels between mammalian p53 and plant SOG.　Because SOG1 and p53 have no amino acid sequence similarity, we propose that plants have evolved a unique system, SOG, instead of p53 to maintain their genome stability.

Here, we analyze the mechanism of SOG1 activation in response to DNA damage to clarify the DNA checkpoint unique to plants.　The results of this study will be discussed.

16:00

The role of cytosolic Chk1 in cell-cycle regulation

Ikuko Tsujimoto

Checkpoint kinase 1 (Chk1) is a key serine/threonine kinase in many cellular responses to genotoxic stress, guarding the integrity of the genome. Chk1 exists not only in the nucleus but also in the cytoplasm. Many studies demonstrated the roles of nuclear Chk1 in cell-cycle checkpoint pathway. However, the significance of cytosolic Chk1 remains to be elucidated. Therefore, we focused on cytoplasmic Chk1 to investigate a Chk1-specific function and then generated stable lines of NIH-3T3 cells that express Chk1-ER (estrogen receptor) fusion protein (Chk1-ER). The subcellular localization of Chk1-ER can be controlled by 4-hydroxytamoxifen (OHT). We recognized that Chk1-ER was kept cytoplasmic, but it was rapidly translocated to the nucleus following OHT treatment.

Chk1-ER expression in cells arrested in G0/G1 by serum deprivation delayed the progression through G1 and to enter S phase. During the progression of G1 phase, the expression of cyclin D1 is increased and subsequently the expressions of cyclin E and cyclinA are induced. These are known as cell-cycle regulatory factors. Chk1-ER led to the delay of their inductions without altering the expression of cyclin D1 mRNA. Using GST pull down assay, we found that Chk1 bound to USP2, which was identified as a specific deubiquitinase for cyclin D1. These results suggested that cytosolic Chk1 might regulate the progression of G1 phase through the inhibition of cyclin D1 expression.