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COMMENTARY

Visualization and orchestration of the dynamic molecular society in cells

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Visualization of specific molecules and their interactions in real space and time is essential to delineate how cellular plasticity and dynamics are achieved and orchestrated as perturbation of cellular plasticity and dynamics is detrimental to health. Elucidation of cellular dynamics requires molecular imaging at nanometer scale at millisecond resolution. The 1st International Conference on Cellular Dynamics and Chemical Biology held in Hefei, China (from 12 September to 15 September, 2008) launched the quest by bringing synergism among photonics, chemistry and biology.

The conference was held in conjunction with the 50th anniversary of the University of Science & Technology of China (USTC). About 100 scientists and students gathered in Hefei to discuss the recent advances in molecular imaging and chemical manipulation of cell biology.

Seeing is believing

The inherent resolution limit of the light microscope has been a hurdle for visualization of molecular nanomachineries in cell biology. Jennifer Lippincott-Schwartz (National Institutes of Health, USA) opened the meeting with a report on the latest supra-resolution imaging technique named PALM (photo-activated localization microscopy) [1]. PALM enables optically imaging intracellular proteins at nanometer (2~25 nm) spatial resolution by isolation of single molecules at high densities (up to ~$10^5/\mu m^2$) based on the serial photo-activation and subsequent bleaching of numerous sparse subsets of photo-activatable fluorescent protein molecules within a sample. By combining PALM with single-particle tracking, the same group developed a new method termed sptPALM [2], which enables the recording of several orders of magnitude more trajectories per cell than traditional single-particle tracking allows. By probing distinct subsets of molecules, sptPALM can resolve the dynamics and origins of spatial and temporal molecular heterogeneities in cells.

Cell division and growth signalling

During cell division, a super-protein structure called kinetochore orchestrates the equal segregation of parental genome into two daughter cells. One of the biggest challenges in the mitosis field is to illustrate the kinetochore assembly dynamics and signaling cascade underlying mitotic checkpoint. Andrea Musacchio of European Institute of Oncology, Italy, has recently succeeded in determining the crystal structure of a truncated "bonsai" version of Ndc80, a tetrameric complex essential for accurate spindle microtubule-chromosome attachment [3]. Xin Cai from Xuebiao Yao's laboratory (USTC, China) presented supporting evidence for the function of Ndc80 in accurate spindle-microtubule attachment regulated by Nek2A-mediated phosphorylation [4]. Andrea Musacchio also presented his working model accounting for the Mad2-dependent spindle checkpoint, in which he proposed that a conformational switch of Mad2 mediates its binding and inhibitory activities towards the Cdc20 activator of the anaphase promoting complex/cyclosome [5].

Cytoplasmic dynein is a minus-end directed microtubule-based molecular motor and plays multiple functions in mitosis. Xueliang Zhu (Shanghai Institutes for Biological Sciences, CAS, China) presented his recent finding in which NudE/Nudel (NudE-like) and Lis1 might serve as novel positive regulators of dynein in eukaryotes [6]. Both the Nudel-Lis1 and Nudel-dynein interactions are crucial for dynein activity. He proposed that Nudel functions not only in dynein-mediated protein transport, but also in stabilizing kinetochore association with dynein

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to facilitate dynein-mediated chromosome poleward movement via tethering to mitosin/CENP-F. To delineate the molecular composition and interaction networks of the mammalian kinetochore, Xuebiao Yao's laboratory carried out a combination of proteomics and systemic yeast 2-hybrid analyses. They revealed the role of Nek2A kinase in orchestrating kinetochore-microtubule attachments and mitotic checkpoint [7, 8]. Recent studies from Yao and colleagues discovered how Nuf2 tethers CENP-E for the kinetochore localization [9], and how septin 7 filaments enable the accurate translocation of CENP-E to the kinetochore [10]. Yao also presented a molecular dissection of the CENP-E interaction network in mitotic checkpoint signalling.

One central question in the cell cycle field is what controls the phase transitions? Although the accumulation of mitotic cyclins drives the transition from the G2 phase to the M phase in embryonic cells, the trigger for mitotic entry in somatic cells remains unknown. Guowei Fang (Stanford University, USA) reported his recent finding that Bora synergizes with the Aurora A kinase in controlling the G2-M transition [11]. Bora accumulates in the G2 phase and promotes Aurora A-mediated activation of Plk1, leading to the activation of cyclin-dependent kinase 1 and mitotic entry. Mechanistically, Bora interacts with Plk1 and controls the accessibility of its activation loop for phosphorylation and activation by Aurora A. While chromosome segregation in mitosis is orchestrated by protein kinase- and phosphatase-signaling cascades, early studies showed that overexpression of human phosphatase Cdc14A, an antagonist of cyclin-dependent kinase 1, affects several aspects of cell division. However, the molecular mechanism underlying HsCdc14A regulation in mitosis has remained elusive. Kai Yuan of Xuebiao Yao's laboratory showed that Cdc14A activity is regulated by an auto-inhibitory mechanism via intra-molecular interaction [12]. Plk1 interacts with and phosphorylates Cdc14A, which releases the intra-molecular interaction of Cdc14A. Using a combination of Plk1 activity reporter and a sensor to monitor Cdc14A intra-molecular interaction, Yuan is profiling the spatiotemporal dynamics of Cdc14A-Plk1 interaction in real-time chromosome segregation.

TGF-β signalling plays pivotal roles in embryogenesis and cancer development. This signalling pathway is spatiotemporally regulated to orchestrate cellular dynamics and homeostasis. Ye-Guang Chen (Tsinghua University, China) presented studies on several newly identified proteins implicated in TGF-β signalling [13, 14]. He showed that Dapper2 negatively regulates TGF-β/Nodal signaling by influencing lysosomal degradation of the endocytosed TGF-β/Nodal receptors while endofin, a member of the FYVE domain protein family, functions in endosomal trafficking. Preliminary evidence suggests that membrane lipid-rafts participate in TGF-β signalling dynamics by determining the activation of MAPK by TGF-β [15]. Systemic acquired resistance (SAR) is a plant immune response that can be induced after a local infection. Using SAR as a model system, Xinnian Dong (Duke University, USA) showed that *Arabidopsis* NPR1 protein is an essential signaling component of SAR. NPR1 is sequestered in the cytoplasm as an oligomer through intermolecular disulfide bonds and S-nitrosylation of NPR1 by S-nitrosoglutathione at cysteine-156 facilitates its oligomerization, suggesting a link between pathogen-triggered redox changes and gene regulation in plant immunity [16].

Structure and activity

One of our current challenges in understanding cellular dynamics is to delineate the structural modules mediating protein-protein interactions and principles underlying the networking of such interactions. This requires an accurate understanding of their points of contact. Yunyu Shi (USTC, China) presented her studies on human PPIL1 (peptidyl prolyl isomerase-like protein 1), a component in 45S U5 snRNP in activated spliceosome B* and 35S snRNP. It was believed to participate in the activation of spliceosome. Using NMR spectroscopic analysis, the solution structure of PPIL1 was determined and shown to resemble that of other members of the cyclophilin family [17]. Chemical shift perturbation experiments were employed to study the interaction between PPIL1 and SKIP (Ski-interaction protein), another essential component of 45S activated spliceosome. Their studies illustrated that a novel cyclophilin-protein contact module exists in the PPIL1-SKIP complex, suggesting that protein-protein contact-mediated conformation changes may function in activation of the spliceosome.

Reversible protein tyrosine phosphorylation plays a major role in cellular signalling by changing protein's plasticity. However, delineation of the functional significance of PTPs in normal physiology and in diseases remains a major challenge in cell signaling as genetic manipulations such as gene knockout and RNAi can not resolve the involvement of a protein in two temporally separated events. Zhong-yin Zhang (Indiana University, USA) described how chemical biology approaches with potent and selective small molecule inhibitors can be used to delineate PTP function and to develop PTP-based therapeutics [*e.g.*, 18]. Annotation of the budding yeast *Saccharomyces cerevisiae* genome revealed that there are 80 genes involved in oxidative stress response. Using a structural genomics approach, Congzhao Zhou and colleagues (USTC, China) revealed the structural basis of protein-mediated electron transfer in response to oxidative stress [19]. The 2b proteins encoded by

cucumovirus act as post-transcriptional gene silencing suppressors to counter host defence during infection. Adma Yuan (Singapore National University, Singapore) reported the crystal structure of 2b protein complexed with a 19 bp small interfering RNA duplex [20]. The 2b protein oligomerizes to form a dimer of dimers through the conserved leucine-zipper-like motif at its amino-terminal alpha-helix.

Chemical biology of enzymes

The family of 14-3-3 proteins functions as critical regulators underlying diverse signaling events in health and diseases. Given the fact that 14-3-3-binding often switches the plasticity of its binding partners, Haian Fu (Emory University, USA) devised a high-throughput screen strategy for identifying chemicals that disrupt the association of 14-3-3 with its binding partners and carried out a large scale screening campaign [21]. Since 14-3-3 is up-regulated in certain type of lung cancers, these 14-3-3 inhibitors are of substantial significance in dissecting spatiotemporal dynamics of 14-3-3 and are also expected to be of great benefit in leading to therapeutic strategies for treating diseases such as cancer.

Polo and Aurora family kinases regulate important events during mitosis including centrosome maturation and separation, mitotic spindle assembly, and chromosome segregation. Mis-regulation of Aurora and Polo kinases due to genetic amplification and protein overexpression results in aneuploidy and may contribute to tumorigenesis. Therefore, chemical inhibitors of Aurora and Polo are currently undergoing phase 2 clinical trials. Jiancun Zhang (Guangzhou Institute of Biomedicine and Health, CAS, China) provided his rationale design of allosteric inhibitors for mitotic regulators such as Mps1.

Autophagy is a lysosome-dependent cellular catabolic mechanism underlying the turnover of intracellular organelles and long-lived proteins. Reduction of autophagy activity has been shown to lead to the accumulation of misfolded proteins in disease settings. Dawei Ma and colleagues (Shanghai Institute of Organic Chemistry, CAS, China) recently discovered some small molecule regulators of autophagy using an image-based screening strategy [22]. Interestingly, these compounds can promote degradation of long-lived proteins and reduce the levels of expanded polyglutamine repeats in cultured cells, raising the possibility that some of these drugs may be useful for the treatment of human diseases associated with the accumulation of misfolded proteins.

During cell division, chromosome segregation is orchestrated by the interaction of spindle microtubules with the centromere. A dramatic remodeling of interpolar microtubules into an organized central spindle between the separating chromatids is required for the initiation and execution of cytokinesis. It becomes increasingly clear that a cohort of kinesin molecules cooperate to orchestrate the spindle dynamics in mitosis. To dissect the spatiotemporal dynamics, Xuebiao Yao and colleagues set up a combination of phenotype-based screen for mis-aligned chromosomes in mitosis and inhibition of CENP-E activity *in vitro*. Treatment of these novel CENP-E inhibitors resulted in chromosome alignment errors, which is reminiscent of siRNA-mediated knockdown of CENP-E. The excitement ahead is to use these valuable chemical tools to dissect the temporal function of CENP-E in mitosis.

Kaixian Chen (Shanghai Institutes for Biological Sciences, CAS, China) exemplified the case study of application of chemical biology strategies for pharmacological lead identification and development of traditional Chinese medicine. He elaborated how molecular dynamics and network information can be used for intervening disease development and progression. Given the differential involvement of certain key molecules in health and diseases, he stressed the importance of allosteric modulators in harnessing disease-associated target activity.

Cellular plasticity and manipulation

Glioblastomas display a hierarchy of neoplastic cells with a restricted population of cells that exhibit extensive self-renewal and the capacity to generate complex tumors that recapitulate parental tumours upon xenotransplantation. Xiao-Fan Wang (Duke University, USA) presented an unexpected excitement in which N-myc downstream-regulated gene 4 (Ndrg4) is critical for the survival of glioblastoma cells. Knockdown of *Ndrg4* induces an increase in apoptosis, indicating that Ndrg4 diverges in function from its closely related tumour suppressive family members in the context of glioblastoma.

Cell-based phenotypic and pathway-specific screens of synthetic small molecules and natural products have historically provided useful chemical ligands to modulate and/or study complex cellular processes. Sheng Ding and colleagues (Scripps Research Institute, USA) discovered small molecules that can be used to selectively regulate stem cell fate and developmental signalling pathways [23, 24]. Such molecules will provide useful tools to dissect stem cell plasticity, and may ultimately contribute to regenerative medicine for tissue repair. Reverberatory activity within neuronal cell assembly is thought to represent the online memory trace in the brain and to serve as an elementary unit in thought process. Using cultured hippocampal neurons as a model system, Guoqiang Bi and colleagues (USTC, China) revealed that activity-induced synaptic plasticity could drive the emergence of reverberatory activity in small networks [25]. It would be of great interest to illuminate the molecular regulatory network underlying the reverberatory activity.

Perspectives

Accurate determination of the spatiotemporal dynamics of interacting proteins within a cell is essential for understanding their biological function. Recent success in the use of two-color PALM further demonstrates the feasibility to directly visualize molecular interactions within cellular structures at the nanometer scale [26]. Given the fact that much of the excitement in the genome-wide analyses of protein-protein interaction networks was recently constructed based on biochemical and computational analyses in different organisms [*e.g.*, 27], one obvious question is to visualize how a "party hub" protein like BRCA1 interacts with multiple partners simultaneously, and whether perturbation of the "party hub" would change the cellular plasticity leading to diseases. In addition, it would be of great interest to examine whether fine-tuning of the spatiotemporal dynamics of specific protein-protein interactions in disease settings is sufficient for the cure. In both cases, small molecules targeting protein-protein interactions will be extremely valuable for illuminating the protein-protein interaction network orchestration in health and correcting aberrant protein-protein interactions in diseases. There is no doubt that consolidation of protein-protein interaction network and circuitry combined with nano-scale illumination of molecular dynamics will enable us to delineate the molecular physiology and pathogenesis of diseases.

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