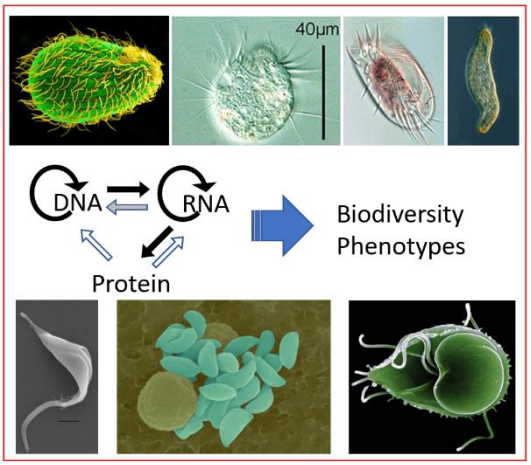
Symposium 2
Molecular and cellular biology of ciliated protozoa

Organizers: Shan Gao (Ocean University of China, China) and De-Hua Lai (Sun Yat-Sen University, China)



Synopsis: Parasitic and free-living protozoans are eukaryotic pathogens and/or biomass of medical and ecological importance. Numerous features of protozoa, such as nuclear dualism, specified cell structure or organelle, distinct cell cycles and phenotypic/genotypic strain diversity, account for their contribution to molecular and cellular biology. Historical highlights of discoveries include telomere and telomerase, ribozyme, histone modifications, microtubule motors, RNA editing, small RNA-directed DNA elimination, and so on. Nonetheless, mechanisms that govern the accuracy of DNA elimination, synaptonemal complex-independent meiosis, stage specific metabolism and regulation, stage differentiation and species diversification remain elusive. This symposium will bring together a diverse group of young researchers interested in revealing molecular and cellular biological machinery of both parasitic and free-living protozoans. A combined tool of conventional and molecular genetics, biochemistry, cytology, and bioinformatics was applied to address how protozoans maintain the genome integrity, survive in competition, and diversify. Many of their findings have much to offer for future studies of protozoans themselves and consequently human-related basic and medical biology.

S2-1

Multi-HP1-like protein containing complex regulates DNA elimination in *Tetrahymena*

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Heterochromatin plays important roles in transposon (TE) silencing. A major type of heterochromatin contains chromatin that is methylated at histone H3, lysine 9/27 (H3K9/27me) and its reader HP1 proteins that recruit diverse proteins onto the chromatin to silence the TEs. Although multiple HP1 proteins are co-expressed in many eukaryotic cells, the interplay between these HP1 proteins has been elusive. Here, we show that subset of the HP1 proteins interact each other and coordinately promote the eliminations of TE-related Internal Eliminated Sequences (IESs) from the somatic genome during macronuclear development in the ciliated protozoan *Tetrahymena thermophila* . We tethered 7 HP1-like proteins individually to the artificially created locus by LexA-LexO system and found that only a subset of HP1-like proteins (Pdd1, Hpl4, Hpl5 and Jub5) induced the elimination of the tethered site. This ectopic DNA elimination was achieved by their chromoshadow domains alone, indicating that the chromoshadow domains of the distinct type of HP1-like proteins can recruit all the proteins that are required for DNA elimination. Immunoprecipitation of Pdd1 specifically enriched the other HP1-like proteins Hpl4/5 and Jub5, each of which was sufficient for the ectopic DNA elimination. The chromodomains of Pdd1 and Jub5, but not Hpl4/5, showed strong affinity to both H3K9me3 and H3K27me3. Altogether, we suggest that multiple HP1-like proteins cooperatively recognize the methylated histones and form a core complex to recruit other effector proteins for DNA elimination. To clarify entire picture of DNA elimination, we are currently analyzing the spatiotemporal dynamics of heterochromatin assembly.

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S2-2

Rab family small GTP in autophagy and life cycle differentiation in *Trypanosoma brucei*

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Cellular differentiation is important for the life cycle and development of both single-cellular and multi-cellular organisms. Hallmarks of cellular differentiation include activation/inactivation of various signaling pathways and remodeling of transcriptional and translational activities. *Trypanosoma brucei* subspecies are causative agents for African Sleeping Sickness in humans and nagana in cattle. Its life cycle alternates between mammalian hosts and tsetse fly vector, with the parasites taking on different forms adapted to different environmental niches. Within the complex life cycle, the slender-to-stumpy differentiation step occurs in mammal blood is of particular interest as a main point of therapeutic intervention for control of these deadly pathogens. At least three pathways have been found to induce slender-to-stumpy differentiation. The best characterized is the most physiological, stumpy inducing factor (SIF)-induced stumpy formation through a quorum sensing mechanism mediated by the uptake of oligopeptides via GPCR homologue GRP89. Activation of *T. brucei* adenosine monophosphate kinase (TbAMPK) via AMP analogs or oxidative stress also induce stumpy formation, possibly via concomitant inhibition of an unconventional Target of Rapamycin homolog, TbTOR4. Recent studies on the expression of variant surface glycoproteins (VSGs) that are crucial for parasite immune evasion also found a link between VSG expression suppression and stumpy formation. While each of these pathways could function during parasite differentiation *in vivo* and together ensure stumpy formation and parasite transmission, it is not clear if there is crosstalk among these pathways, and whether or how these different pathways all converge to the same cell fate determinants leading to stumpy formation. In our recent studies to examine the role of small GTPases in autophagy, we identified TbRab2B as an autophagy regulator, functioning late at the autophagosome degradation step. TbRab2B is normally present at the Golgi apparatus in both procyclic and slender form cells under fed conditions, but relocates to the lysosomes under amino acid-starvation conditions, possibly as activated GTP-bound form. Further characterization revealed a role of TbRab2B in lysosome biogenesis and function in both procyclic and slender form cells. Most intriguingly, depletion of TbRab2B in the monomorphic slender cells induced cell differentiation to the stumpy form, which could be further differentiated to stable procyclic cells. Together these results strongly supported a novel function of TbRab2B as a negative regulator of slender-to-stumpy differentiation. Whether TbRab2B is part of the known pathways or represents a new differentiation mechanism awaits further studies.

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S2-3

RNAi-dependent Polycomb repression controls transposable elements in *Tetrahymena*

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RNAi and Polycomb repression play evolutionarily conserved and often coordinated roles in transcriptional silencing. Here, we show that, in the protozoan *Tetrahymena thermophila*, germline-specific internally eliminated sequences (IESs)—many related to transposable elements (TEs)—become transcriptionally activated in mutants deficient in the RNAi-dependent Polycomb repression

pathway. Germline TE mobilization also dramatically increases in these mutants. The transition from noncoding RNA (ncRNA) to mRNA production accompanies transcriptional activation of TE-related sequences and vice versa for transcriptional silencing. The balance between ncRNA and mRNA production is potentially affected by cotranscriptional processing as well as RNAi and Polycomb repression. We posit that interplay between RNAi and Polycomb repression is a widely conserved phenomenon, whose ancestral role is epigenetic silencing of TEs.

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S2-4

Adaption and applications of plant auxin inducible degron systems in *Toxoplasma gondii*

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Toxoplasma gondii is an obligate and intracellular parasite, which infects almost all warm-blooded animals. Though *T. gondii* is a good model for studying apicomplexan parasites due to its ease of culturing and genetic traceability, the genetic modification and downregulation of proteins are not as efficient and prompt as expected for better dissection of proteins function, using the bacterial tetracycline operator/regulator system and dimerizable Cre-recombinase system. In recent years, we introduced the plant-derived auxin-inducible degron (AID) system I and II into *T. gondii*, by applying the rational in which plant auxin (IAA) can bind TIR1 and activate the ubiquitin proteasome system to degrade AID degron fusion proteins. We efficiently dissected the function of conoid hub protein 1 (CPH1) on conoid stability and parasite motility by combining CRISPR technology and the AID system I. In the second AID system (AID II), we adapted the upgraded system in which a mutation at residue 74 (F74G) of TIR1 makes the TIR1 to use a bump-and-hole approach to improve the performance of protein degradation system. A bumped-IAA analogue 5-Ph-IAA showed high efficiency of induction using a much lower level of inducer (1 nM), comparing to the IAA (500 nM). The AID II system offers another advantage of using a 1/3 length of the original AID degron – miniAID. Recently we have successfully applied the AID II system to study a key phosphatase possibly regulating parasite cytoskeleton, and a novel protein involved in parasite division. The AID I and II system will be highly useful for studying *T. gondii* and other related parasites, to promote basic study and pharmaceutical development.

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S2-5

A feedback mechanism controls meiotic DNA double-strand break formation in *Tetrahymena*

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Meiosis is a special cell division programme for producing gametes (i.e. eggs and sperm). Successful meiotic recombination is ensured by the programmed induction of DNA double-strand breaks (DSBs), which are the most dangerous form of DNA damage. Thus, the DSB number is strictly controlled because they are potentially harmful. We found a novel protein, Pars11, which is required for Spo11-dependent DSB formation in the protest *Tetrahymena*. Pars11 localizes to chromatin early in meiotic prophase in a Spo11 independent manner and is removed before the end of prophase. Pars11 removal depends on DSB formation and ATR-dependent phosphorylation. In the absence of the ATR, a DNA damage sensor kinase, Pars11 is retained on chromatin and excess DSBs are generated. Similar levels of Pars11 persistence and DSB overproduction occur in a non-phosphorylatable pars11 mutant. We conclude that Pars11 supports DSB formation by Spo11 until enough DSBs are formed; thereafter, DSB production stops in response to ATR-dependent degradation of Pars11 or its removal from chromatin. A similar DSB control mechanism involving a Rec114-Tel1/ATM-dependent negative feedback loop regulates DSB formation in budding yeast. However, there is no detectable sequence homology between Pars11 and Rec114, and DSB numbers are more tightly controlled by Pars11 than by Rec114. The discovery of this mechanism for DSB

regulation in the evolutionarily distant protist and fungal lineages suggests that it is conserved across eukaryotes.

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S2-6

Cyclic Nucleotide Signaling in the Obligate Intracellular Pathogen *Toxoplasma gondii*

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Infection, pathogenesis and transmission of the intracellular parasitic protist, *Toxoplasma gondii*, depend on cAMP, cGMP and calcium signaling. Cyclic nucleotide cascades in this widespread model pathogen show a remarkable parasite-specific divergence compared to mammalian host cells. Our group has implemented wide-ranging methods including genome engineering and mutant phenotyping to determine the physiological importance of cAMP and cGMP during lytic cycle (acute infection) of *T. gondii*. In extended work, we deployed optogenetics in conjunction with phosphoproteomics and gene mutagenesis to identify novel signaling mediators and decipher the hierarchical topology of cyclic nucleotide cascades. Not least, our research has pioneered the utility of light-activated proteins for dynamic, reversible, specific and spatiotemporal control of cAMP and cGMP in genetically-tractable pathogens.

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