董云伟团队关于蛋白质温度适应性变化的研究成果再次在《美国科学院院报》（PNAS）发表

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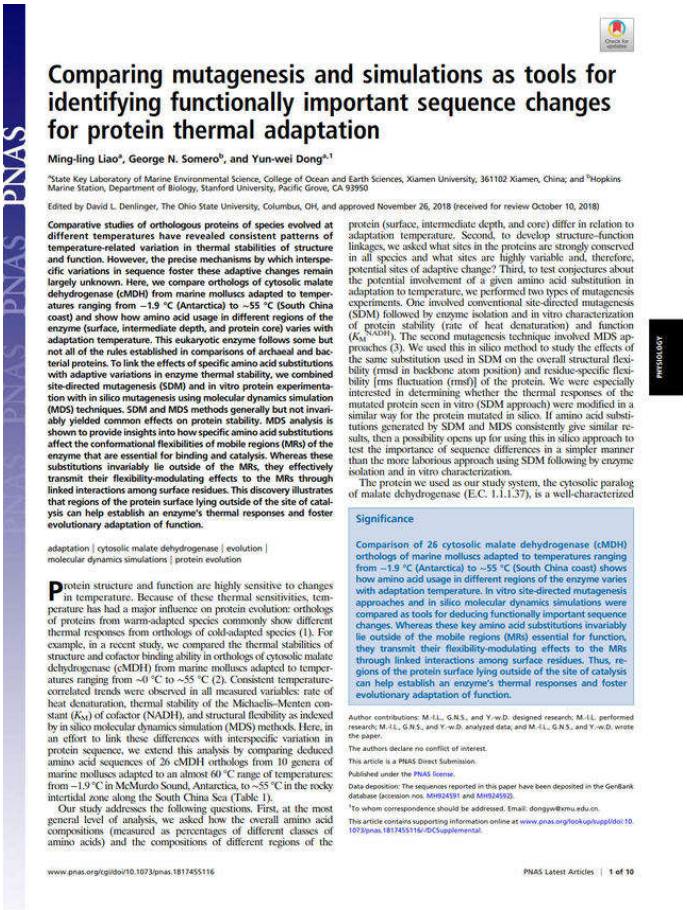
12月24日，我校近海海洋环境科学国家重点实验室、海洋与地球学院董云伟教授与斯坦福大学George Somero 教授合作，在 Proceedings of the National Academy of Sciences of the United States of America (PNAS)（《美国科学院院报》）发表题为“Comparing mutagenesis and simulations as tools for identifying functionally important sequence changes for protein thermal adaptation”的研究论文，探讨了海洋软体动物蛋白质温度适应性变化模式。这一文章与该团队2018年初在PNAS发表的“Structural flexibility and protein adaptation to temperature: Molecular dynamics analysis of malate dehydrogenases of marine mollusc”一文共同开辟了利用计算生物学进行贝类进化研究的方向。



Comparing mutagenesis and simulations as tools for identifying functionally important sequence changes for protein thermal adaptation

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Comparative studies of orthologous proteins of species evolved at different temperatures have revealed consistent patterns of temperature-related variation in thermal stabilities of structure and function. However, the precise mechanisms by which interspecific variations in sequence foster these adaptive changes remain largely unknown. Here, we compare orthologs of cytosolic malate dehydrogenase (cMDH) from marine molluscs adapted to temperatures ranging from −1.9 °C (Antarctica) to ~55 °C (South China coast) and show how amino acid usage in different regions of the enzyme (surface, intermediate depth, and protein core) varies with adaptation temperature. This eukaryotic enzyme follows some but not all of the rules established in comparisons of archaeal and bacterial proteins. To link the effects of specific amino acid substitutions with adaptive variations in enzyme thermal stability, we combined site-directed mutagenesis (SDM) and in vitro protein experimentation with in silico mutagenesis using molecular dynamics simulation (MDS) techniques. SDM and MDS methods generally but not invariably yielded common effects on protein stability. MDS analysis is shown to provide insights into how specific amino acid substitutions affect the conformational flexibilities of mobile regions (MRs) of the enzyme that are essential for binding and catalysis. Whereas these substitutions invariably lie outside of the MRs, they effectively transmit their flexibility-modulating effects to the MRs through linked interactions among surface residues. This discovery illustrates that regions of the protein surface lying outside of the site of catalysis can help establish an enzyme's thermal responses and foster evolutionary adaptation of function.

adaptation | cytosolic malate dehydrogenase | evolution | molecular dynamics simulations | protein evolution

Protein structure and function are highly sensitive to changes in temperature. Because of these thermal sensitivities, temperature has had a major influence on protein evolution: orthologs of proteins from warm-adapted species commonly show different thermal responses from orthologs of cold-adapted species (1). For example, in a recent study, we compared the thermal stabilities of structure and cofactor binding ability in orthologs of cytosolic malate dehydrogenase (cMDH) from marine molluscs adapted to temperatures ranging from ~0 °C to ~55 °C (2). Consistent temperature-correlated trends were observed in all measured variables: rate of heat denaturation, thermal stability of the Michaelis–Menten constant (K_M) of cofactor (NADH), and structural flexibility as indexed by in silico molecular dynamics simulation (MDS) methods. Here, in an effort to link these differences with interspecific variation in protein sequence, we extend this analysis by comparing deduced amino acid sequences of 26 cMDH orthologs from 10 genera of marine molluscs adapted to an almost 60 °C range of temperatures: from −1.9 °C in McMurdo Sound, Antarctica, to ~55 °C in the rocky intertidal zone along the South China Sea (Table 1).

Our study addresses the following questions. First, at the most general level of analysis, we asked how the overall amino acid compositions (measured as percentages of different classes of amino acids) and the compositions of different regions of the protein (surface, intermediate depth, and core) differ in relation to adaptation temperature. Second, to develop structure–function linkages, we asked what sites in the proteins are strongly conserved in all species and what sites are highly variable and, therefore, potential sites of adaptive change? Third, to test conjectures about the potential involvement of a given amino acid substitution in adaptation to temperature, we performed two types of mutagenesis experiments. One involved conventional site-directed mutagenesis (SDM) followed by enzyme isolation and in vitro characterization of protein stability (rate of heat denaturation) and function (K_M^{NADH}). The second mutagenesis technique involved MDS approaches (3). We used this in silico method to study the effects of the same substitution used in SDM on the overall structural flexibility (rmsd in backbone atom position) and residue-specific flexibility [rms fluctuation (rmsf)] of the protein. We were especially interested in determining whether the thermal responses of the mutated protein seen in vitro (SDM approach) were modified in a similar way for the protein mutated in silico. If amino acid substitutions generated by SDM and MDS consistently give similar results, then a possibility opens up for using this in silico approach to test the importance of sequence differences in a simpler manner than the more laborious approach using SDM following by enzyme isolation and in vitro characterization.

The protein we used as our study system, the cytosolic paralog of malate dehydrogenase (E.C. 1.1.1.37), is a well-characterized

Significance

Comparison of 26 cytosolic malate dehydrogenase (cMDH) orthologs of marine molluscs adapted to temperatures ranging from −1.9 °C (Antarctica) to ~55 °C (South China coast) shows how amino acid usage in different regions of the enzyme varies with adaptation temperature. In vitro site-directed mutagenesis approaches and in silico molecular dynamics simulations were compared as tools for deducing functionally important sequence changes. Whereas these key amino acid substitutions invariably lie outside of the mobile regions (MRs) essential for function, they transmit their flexibility-modulating effects to the MRs through linked interactions among surface residues. Thus, regions of the protein surface lying outside of the site of catalysis can help establish an enzyme's thermal responses and foster evolutionary adaptation of function.

Author contributions: M.-l.L., G.N.S., and Y.-w.D. designed research; M.-l.L. performed research; M.-l.L., G.N.S., and Y.-w.D. analyzed data; and M.-l.L., G.N.S., and Y.-w.D. wrote the paper.

The authors declare no conflict of interest.

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. MH924591 and MH924592).

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董云伟团队在潮间带生物生化适应机制的研究过程中，结合分子动力学模拟和实验调控手段，发现极端高温下，耐热滨螺能够通过增强代谢关键酶的作用，避免蛋白质的解链，保持微结构完整和功能维持（J Exp Biol, 2017）；通过对原位体温跨度达60℃的12种软体动物的研究，定量了cMDH结构柔性的温度适应性变化程度，揭示了氨基酸温度适应性进化的关键位点，阐述了蛋白质结构稳定性与生物地理分布的内在联系。将海洋软体动物生化适应研究从单一的定性实验，拓展到了基于计算生物学的定量研究，揭示了海洋软体动物细胞质苹果酸脱氢酶（cMDH）结构稳定性和功能适应性的趋同进化模式，建立了基于代谢关键酶的“酶促动力学—蛋白合成—模拟计算”的生化适应机制的创新性研究模式。

基于上述基础，课题组进一步拓展研究的深度与广度，比较分析了从南极洲半致死温度仅为 4 ° C 的扇贝，到中国沿海可耐受 60 ° C 以上高温的滨螺等26种海洋软体动物 cMDH 的温度耐受性，提出了蛋白质不同区域氨基酸的温度适应性变化模式，通过分子动力学分析揭示了具有重要功能的区域及其作用机制。这一系列研究成果加深了对海洋生物蛋白质温度适应机制的认识，为该领域提供了新的研究模式与思路，对于查明环境温度对生物分布的影响及其机制，预测气候变暖的生态学效应具有重要意义。

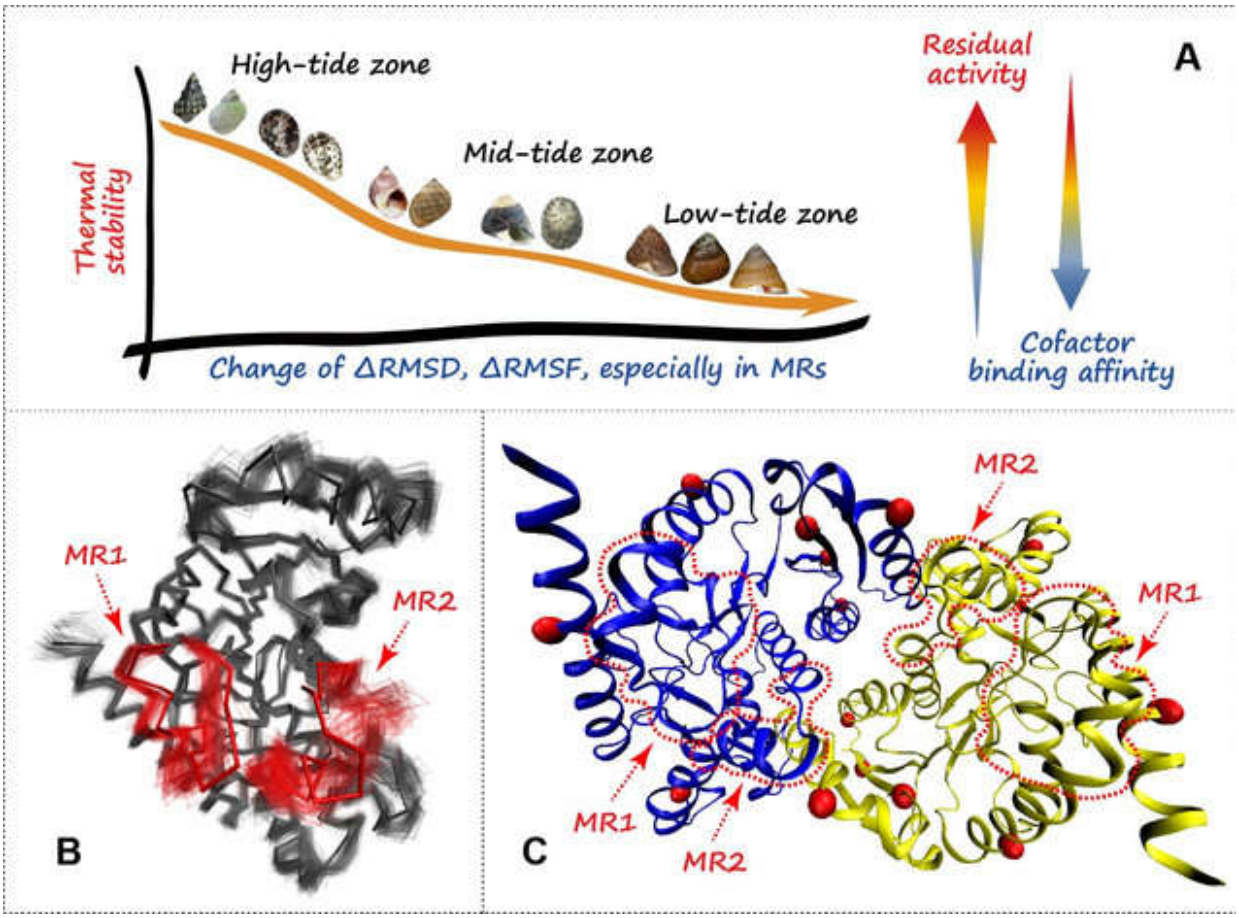

图. (A) 具有不同水平和垂直分布、原位温度迥异的海洋软体动物具有不同的热耐受性，并且与 cMDH 结构刚性和柔性的变化程度负相关；(B) 分子动力学模拟（MDS）高温变性过程中，主要刚性和柔性的变化发生在柔性区域（MR），实线为粒结节滨螺（Echinolittorina radiata）cMDH 初始结构，虚线为 57 ° C 下，0-2ns 结构变性的变化轨迹； (C) 塔结节滨螺（E. malaccana）的二聚体结构，红色球体为E. malaccana, E. radiata, Littorina keenae 和 L. scutulata cMDH非保守替代位点，氨基酸变异位点总是位于 MRs 外，四者具备高度保守的序列，但热耐受性迥异。

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董云伟团队致力于潮间带生态学研究，重点研究潮间带生物对复杂环境条件的响应特征和时空规律，及其适应机制。近年来研究主要集中在潮间带生物生化适应机制、生理调节策略及地理格局变化等方面。（1）生理调节策略方面：建立了以能量代谢和应激反应为主要参数的生理响应模型，阐释了温度和降水等多重环境胁迫影响潮间带种群动态的机制（Funct Ecol，2016；Mol Ecol，2014）。整合环境和生理数据，查明我国潮间带生物对温度变化的敏感性及其纬度特征（P Roy Soc B，2017）。与国外合作者共同发表评述提出要重视海洋多重尺度环境变化的生态效应（Nature，2018）。（2）地理格局变化方面：确定了我国潮间带软体动物存在着以长江口为界的生物地理格局，提出了海堤修建会导致生物分布区迁移（Science，2015）；证实了沿岸建筑已成为我国潮间带生物扩散的“跳板”，减弱了长江口原有的隔离效应，促进了南北群体间的交流，改变了潮间带生物地理分布格局（Divers Distrib，2016）；首次发现了气候变化和人类活动造成我国潮间带软体动物向北迁移的证据。

论文原文链接：https://www.pnas.org/content/early/2018/12/19/1817455116

董云伟教授个人主页：https://mel.xmu.edu.cn/teacherfile.asp?tid=353

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