NMT
——科研路上好帮手

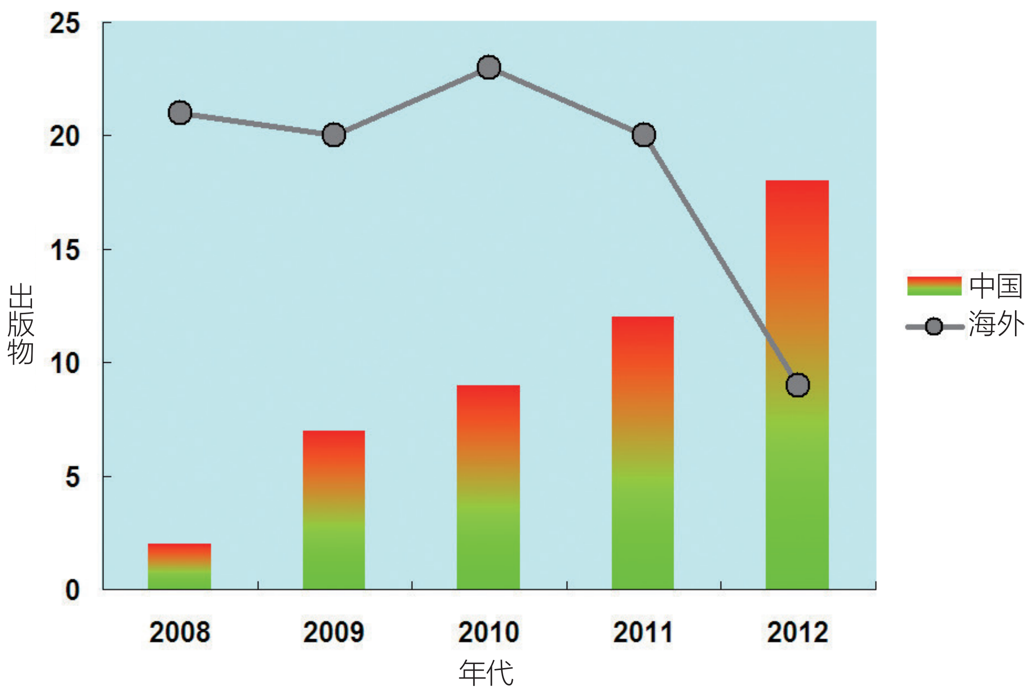
撰文　杨明

非损伤微测技术(Non-invasive Micro-test Technique, NMT)通过实时测定活细胞的离子和分子流速来揭示生命的功能，是后基因组学时代研究信号传递的最佳技术之一。

NMT在生理状态下测定活细胞的指标，同时具有高时间分辨率和空间分辨率，能够测定到细胞实时的流速，不仅反应测定指标的质变，而且还能反应细胞的量变，获取流速的动态曲线，从中可能得到细胞的节律或者振荡规律，这是传统技术无法比拟的优势。通过这项技术，澳大利亚的Sergey Shabala教授已经得到了小麦根部离子流的振荡规律这一重大的发现。

科研人员通过NMT测定绿藻的氧气(O_2)流速发现，绿藻在光暗条件转换之下具有明显的O_2流速的转换，即从外流转变为内流，出现符合正弦曲线的振荡规律。在黑暗中长时间生长，O_2流速的振幅下降，逐渐趋近于零。但是，在这个过程中，绿藻周围的氧气浓度($[O_2]$)却保持较为稳定的状态，即$[O_2]$没有发生变化。

这说明，绿藻在光照条件下



进行光合作用，释放大量的O_2，逐渐达到放氧的平衡。在黑暗中，O_2外流快速下降，转变为耗氧（内流或者呼吸）。长时间处于黑暗中，绿藻没有获得外部的能量，呼吸能力下降，逐渐进入休眠或者步入死亡。因此，只有通过O_2流速才能反映出如此重要的生命活动过程，仅仅测定浓度的方法则无法获知。

在这个实验中，NMT直接测定了光暗中的O_2变化来反应绿藻的光合和呼吸作用，清晰地表现了绿藻的生长受到光调控的这一事实。其中O_2流速的振荡和转换是光调控生长的重要指标，有助于我们便捷地了解绿藻的状态。

使用传统的测定方法，有诸多不利因素限制了这个实验的进行，例如，荧光方法难以同时在光暗下测定；光合作用仪难以测定水生材料和体积很小的材料；气体和元素分析法在时间和空间分辨率上远远不及，且无法测定活细胞的指标。因此，NMT的特点使得我们能够实时、快速、长时间地知道活细胞发生的情况，这些情况包括其特殊的结果，例如振荡规律（节律）、灵敏度高的离子和分子流速等。

综上所述，NMT通过测定离子和分子的流速直观地反应生命的功能，传统方法和仅仅测定浓度的方法难以获得活细胞的节律变化。因此，我们使用NMT将可能揭开特殊的生命奥秘，例如绘制真实的营养吸收曲

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The *Arabidopsis* Chaperone J3 Regulates the Plasma Membrane H⁺-ATPase through Interaction with the PKS5 Kinase

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The plasma membrane H^+-ATPase (PM H^+-ATPase) plays an important role in the regulation of ion and metabolite transport and is involved in physiological processes that include cell growth, intracellular pH, and stomatal regulation. PM H^+-ATPase activity is controlled by many factors, including hormones, calcium, light, and environmental stresses like increased soil salinity. We have previously shown that the *Arabidopsis thaliana* Salt Overly Sensitive2-Like Protein Kinase5 (PKS5) negatively regulates the PM H^+-ATPase. Here, we report that a chaperone, J3 (DnaJ homolog 3; heat shock protein 40-like), activates PM H^+-ATPase activity by physically interacting with and repressing PKS5 kinase activity. Plants lacking *J3* are hypersensitive to salt at high external pH and exhibit decreased PM H^+-ATPase activity. J3 functions upstream of *PKS5* as double mutants generated using *j3-1* and several *pks5* mutant alleles with altered kinase activity have levels of PM H^+-ATPase activity and responses to salt at alkaline pH similar to their corresponding *pks5* mutant. Taken together, our results demonstrate that regulation of PM H^+-ATPase activity by J3 takes place via inactivation of the PKS5 kinase.

北京生命科学研究所的郭岩实验室使用非损伤微测技术在活体拟南芥的根部进行了原位测量，证实不同的基因所调控的质子外流（H^+efflux）的差异，说明了蛋白之间的相互作用。本研究发现，分子伴侣蛋白J3可以和PKS5相互作用，并且抑制PKS5激酶活性，进而正向调节质膜H^+-ATPase的活性及植物对盐碱胁迫的反应

相关研究成果发表在2010年的*Plant Cell*上

线，发现光合作用和呼吸作用的规律，了解信号传递的先后顺序和快慢等。

NMT在中国经过5年的发展，从2008年发表2篇文章，到2012年9月共发表48篇文章，文章数量逐年递增。尤其在2012年里，中国科学家发表的NMT文章总数（18篇）已经超过了国外的文章总数（9篇）。很明显，NMT研究的中心逐渐从国外转移到中国，中国已经成为NMT研究成果产出的主要地区。

这样的变化离不开旭月（北京）科技有限公司和美国扬格公司在NMT发展中所作出的贡献，正是这两家企业的努力，把NMT种植在中国，发展出了适合中国科学家的研究平台和商业模式，并且与北京林业大学、中国科学院植物研究所等单位合作，快速推动了NMT在中国的应用。

NMT在中国从无到有，从有到结出丰硕的成果，我们把NMT的优势转化成了中国科研的成果与实力，这正是NMT的魅力所在。■

（责编　王华）

2012年湖南大学曾光明、陈桂秋实验室在环境科学领域的顶级杂志*Environmental Science & Technology*上发表文章。本文使用非损伤微测技术直接测定了真菌的生理变化，而生理变化通常是细胞对环境反应的最早事件，这为环境的预警和评价提供了非常有价值的工具，因此，NMT可用于环境的预警和评价。这篇文章是中国学者使用非损伤微测技术在环境领域的杂志发表的最高水平的文章。本文的流速数据全部在旭月公司的测试中心（www.xuyue.net）获得

Environmental Science & Technology
Article
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Responses of *Phanerochaete chrysosporium* to Toxic Pollutants: Physiological Flux, Oxidative Stress, and Detoxification

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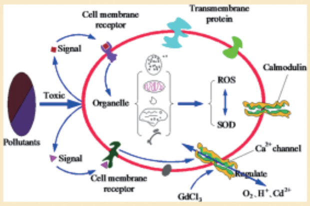
Supporting Information

ABSTRACT: The white-rot fungus *Phanerochaete chrysosporium* has been widely used for the treatment of waste streams containing heavy metals and toxic organic pollutants. The development of fungal-based treatment technologies requires detailed knowledge of the relationship between bulk water quality and the physiological responses of fungi. A noninvasive microtest technique was used to quantify real-time changes in proton, oxygen, and cadmium ion fluxes following the exposure of *P. chrysosporium* to environmental toxic (2,4-dichlorophenol and cadmium). Significant changes in H^+ and O_2 flux occurred after exposure to 10 mg/L 2,4-dichlorophenol and 0.1 mM cadmium. Cd^{2+} flux decreased with time. Reactive oxygen species formation and antioxidant levels increased after cadmium treatment. Superoxide dismutase activity correlated well with malondialdehyde levels (r^2 = 0.964) at low cadmium concentrations. However, this correlation diminished and malondialdehyde levels significantly increased at the highest cadmium concentration tested. Real-time microscale signatures of H^+, O_2, and Cd^{2+} fluxes coupled with oxidative stress analysis can improve our understanding of the physiological responses of *P. chrysosporium* to toxic pollutants and provide useful information for the development of fungal-based technologies to improve the treatment of wastes cocontaminated with heavy metals and organic pollutants.



Combined Proteomic and Cytological Analysis of Ca^{2+}-Calmodulin Regulation in *Picea meyeri* Pollen Tube Growth[1[C][W]]

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Ca^{2+}-calmodulin (Ca^{2+}-CaM) is a critical molecule that mediates cellular functions by interacting with various metabolic and signaling pathways. However, the protein expression patterns and accompanying serial cytological responses in Ca^{2+}-CaM signaling deficiency remain enigmatic. Here, we provide a global analysis of the cytological responses and significant alterations in protein expression profiles after trifluoperazine treatment in *Picea meyeri*, which abrogates Ca^{2+}-CaM signaling. Ninety-three differentially displayed proteins were identified by comparative proteomics at different development stages and were assigned to different functional categories closely related to tip growth machinery. The inhibition of Ca^{2+}-CaM signaling rapidly induced an increase in extracellular Ca^{2+} influx, resulting in dramatically increased cytosolic Ca^{2+} concentrations and ultrastructural abnormalities in organelles as the primary responses. Secondary and tertiary alterations included actin filament depolymerization, disrupted patterns of endocytosis and exocytosis, and cell wall remodeling, ultimately resulting in perturbed pollen tube extension. In parallel with these cytological events, time-course experiments revealed that most differentially expressed proteins showed time-dependent quantitative changes (i.e. some signaling proteins and proteins involved in organelle functions and energy production changed first, followed by alterations in proteins related to cytoskeletal organization, secretory pathways, and polysaccharide synthesis). Taken together, Ca^{2+}-CaM dysfunction induced serial cytological responses and temporal changes in protein expression profiles, indicating the pivotal role of Ca^{2+}-CaM in the regulation of tip growth machinery.

中国科学院植物研究所林金星研究组使用电镜技术、蛋白质组学技术、荧光技术结合非损伤微测技术提供了全面的结果，分析了CaM的功能和蛋白表达模式

相关成果发表在2009年2月的*Plant physiology*上

Plant, Cell & Environment
Plant, Cell and Environment (2012) doi: 10.1111/j.1365-3040.2012.02497.x

TFT6 and *TFT7*, two different members of tomato 14-3-3 gene family, play distinct roles in plant adaption to low phosphorus stress

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2012年6月，中国科学院南京土壤研究所施卫明实验室与香港中文大学张建华实验室联合发表文章在国际知名植物学期刊*Plant, Cell and Environment*上，发现了14-3-3蛋白家族中的TFT6 和TFT7具有调节植物忍耐低磷（LP）胁迫的作用，阐述了这种调节作用的机理

这项工作使用非损伤微测技术测定了拟南芥根部的H^+流速，发现TFT超表达的植物在LP胁迫下增加H^+的流速和质膜H^+-ATPase的活性

这篇文章是使用非损伤微测技术研究植物应对非生物胁迫的范例，即从基因到蛋白，再到生理功能的一系列工作，清晰地阐明了基因的功能

Plant, Cell & Environment
Plant, Cell and Environment (2012) **35**, 893–916 doi: 10.1111/j.1365-3040.2011.02461.

An ATP signalling pathway in plant cells: extracellular ATP triggers programmed cell death in *Populus euphratica*

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2011年，北京林业大学的陈少良教授实验室使用非损伤微测技术、激光共聚焦显微镜等方法研究了eATP诱导的PCD信号转导途径，研究阐述了胡杨PCD中的ATP信号途径，建立了eATP诱导PCD的模型，为进一步认识ATP的信号网络提供了必不可少的证据

相关成果发表在2011年的*Plant, Cell & Environment*上