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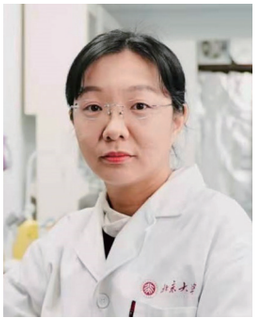

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气孔研究进展：从保卫细胞到光合作用

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摘　要：气孔是由植物表皮上的一对保卫细胞所构成的微孔隙结构，是陆生植物与周围环境进行气体交换的最主要通道。气孔的运动和发育决定着气孔对于气体 (特别是二氧化碳和水汽) 的通导能力，进而影响植物的光合与生长。本综述首先回顾关于气孔运动和发育调控机制的研究，之后介绍气孔与光合作用相关的理论研究进展，最后对通过优化气孔性状提升植物光合速率、生物量与产量的重要成果进行概述，并对今后气孔研究的重要内容进行展望。

关键词：气孔保卫细胞；光合作用；气孔运动；气孔发育；生物量
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Research progress of stomata: from guard cells to photosynthesis

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Abstract: Stomata, composed by a pair of guard cells, are micropores on the epidermis of plants. They are serving as the primary channels for the gas-exchange of terrestrial plants. Both stomatal movement and development determine the ability of stomatal gas conductance (especially for carbon dioxide and water vapor), and thus affect plant photosynthesis and growth. This review first discusses the mechanisms of stomatal movement and development regulation, then introduces the advances in research on the relationship between stomata and photosynthesis. Furthermore, we summarize the research on optimizing stomatal traits to enhance plant photosynthetic rates and biomass or crop yield. Finally, we prospect the essential topics of stomatal research in the future.

Key words: stomatal guard cells; photosynthesis; stomatal movement; stomatal development; biomass

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气孔 (stoma，常用复数形式 stomata) 主要存在于陆生植物叶片的表面，是由表皮上一对被称为“保卫细胞”(guard cell) 的特殊薄壁细胞所组成的微孔隙结构。约 4 亿年前，气孔的出现使植物表皮既可以保水又可以进行必要的气体交换，推动了维管植物的大规模登陆，是植物进化历程中的一个重要转折点 [1-2]。在大多数情况下，虽然气孔面积仅占叶表面积的 0.3%~5%，但陆生植物与外界环境约 95% 的气体交换都需要通过气孔进行 [3]。

植物的形态虽然千姿百态，但是目前已知的气孔保卫细胞的形态只有两类。一类是肾形保卫细胞 (kidney-shaped guard cell)，存在于双子叶植物和多数单子叶植物中，这类保卫细胞在表皮中分布得比较随机；而在禾本科 (Poaceae)、莎草科 (Cyperaceae)、灯心草科 (Juncaceae) 植物中存在另一类平行排列的保卫细胞，即哑铃形保卫细胞 (dumbbell-shaped guard cell)，在每个保卫细胞外侧还有一个副卫细胞 (subsidiary cell) 协助保卫细胞控制气孔的开闭 [4-5]。

气孔通导气体的能力对于植物的光合与生长至关重要。通常，当气体经由气孔孔隙进行扩散时，会受到来自保卫细胞的阻力，这个阻力称为气孔阻力 (stomatal resistance)。现在的研究中更多地使用气孔阻力的倒数，即气孔导度 (stomatal conductance, g_s)，表征植物与外界进行气体交换的通量。气孔导度的大小取决于气孔运动和气孔发育的情况。大多数研究一般使用气孔开度 (stomatal aperture) 表征保卫细胞体积变化引起的气孔运动；用单位叶面积上的气孔数量，即气孔密度 (stomatal density/frequency)，或气孔指数 (stomatal index)，表征气孔的发育情况。

气孔研究已经有一百多年的历史，特别是本世纪内，随着研究技术的飞速发展，在气孔的分子调控机制方面取得了丰硕的研究成果。本文将从气孔运动与气孔发育的分子调控机制引入，进而延伸到叶片上下两面气孔的差异、叶肉光合作用对气孔运动的调控、不同光合代谢途径植物的气孔差异等一系列气孔与光合作用相关的理论研究。最后，本文简单总结了通过优化气孔性状提高光合速率、水分利用效率，及生物量或作物产量方面的突出成果，并对今后气孔研究的重要课题进行了展望。

1 气孔运动的调控

气孔保卫细胞能够快速感知外界环境的变化，并通过复杂的信号转导机制调控气孔的运动，为植物能够在各种环境条件下平衡光合作用与水分散失提供了重要保障 [6–8]。本文将从气孔运动响应光照、水分、CO_2 浓度、温度的调控机制这四个方面，回顾近几十年的重要研究成果。

1.1 光照

光作为光合作用的能量来源，也是气孔响应外界信号中最重要的环境因子。早在 19 世纪末，Francis Darwin 就已发现不同波长的光照射引起气孔开放的程度存在差异，其中蓝光最容易引起气孔的开放 [9]。后续的研究进一步发现，蓝光可以在低光强下快速引起气孔开放，其效率可达红光的 10 倍以上 [10]。

蓝光诱导的气孔开放在离体的表皮和保卫细胞原生质体中均已得到证实 [11]，说明保卫细胞可以直接感知蓝光，此过程不依赖叶肉的光合作用 [12]。目前已知的主要的蓝光信号转导过程为：蓝光受体向光素 (phototropins, PHOT1 和 PHOT2) 最先感知蓝光 [13-14]，通过自磷酸化启动下游的级联反应，激活保卫细胞质膜的 H^+-ATPase (PM H^+-ATPase)，导致质膜超极化 (hyperpolarization of the plasma membrane)，进而激活内向整流型 K^+ 通道 (inward-rectifying K^+ channels)，引起 K^+ 离子向保卫细胞内聚集 [13,15-17]。K^+ 离子与其他离子 (比如 Cl^- 离子、NO_3^- 离子等) 汇聚到保卫细胞的液泡中 [18-19]，提高了液泡的渗透势，使得液泡吸水膨胀，拉动保卫细胞内侧细胞壁向外运动，开放气孔孔隙。近年的研究进一步表明，保卫细胞细胞质中存在的蛋白激酶 BLUS1(blue light signaling 1) 可被 PHOT1 与 PHOT2 磷酸化激活 [20]，并将信号间接传递给 1 型蛋白磷酸酶 (type 1 protein phosphatase, PP1) 及其调控亚基 PRSL1[20-23]，之后通过磷酸化 14-3-3 蛋白进一步促进质膜 H^+-ATPase 的活化 [15,24-25]。最近的研究还鉴定了一种 Raf-like 蛋白激酶 BHP (blue light-dependent H^+-ATPase PHOSP-Horylation)，它可与 BLUS1 结合间接介导质膜 H^+-ATPase 的磷酸化 [26]。但是目前仍未鉴定到可以直接激活质膜 H^+-ATPase 的激酶 [16,26]。

红光也可以引起气孔的开放，但主要与光合作用或脱落酸 (abscisic acid, ABA) 有关。很多研究表明气孔的红光响应可被光系统 II 电子传递抑制剂 DCMU (N-(3,4- 二氯苯基)N',N'- 二甲基脲) 强烈抑制 [27-29]。虽然对整叶片照射红光可以诱导保卫细胞上的质膜 H^+-ATPase 磷酸化，引起气孔开放，但是当红光直接照射离体的表皮时，气孔并不打开，也无法在保卫细胞中检测到质膜 H^+-ATPase 的磷酸化 [30]，因此，普遍认为叶肉光合作用参与了气孔对

红光的响应过程 (见第 4 章节)[31]。另外，近些年还有研究表明，光敏色素 (phytochrome, Phy) 及其下游转录因子 PIFs (phytochrome interacting factors) 能够参与 ABA 的合成，进而调控气孔对红光的响应 [32-36]，使气孔运动对红光的响应与水分胁迫 (见第 1.2 章节) 建立了联系。

绿光是在叶片下表面和林下冠层中最主要的光质 [37-38]。有研究表明绿光也可以诱导气孔开放 [39-41]，特别是在使用 DCMU 抑制叶片光合的条件下，绿光仍然可以部分诱导向日葵 (*Helianthus annuus*) 叶片下表面气孔的开放 [40-41]，说明在保卫细胞中绿光可能参与类似于蓝光的不依赖光合作用的信号转导过程。进一步，对拟南芥 (*Arabidopsis thaliana*) 向光素突变体 (*phot1phot2*) 的研究表明，保卫细胞感知绿光与感知蓝光的机制不同，可能有一种尚未发现的绿光受体存在于保卫细胞中，介导绿光诱导气孔开放的过程 [31]。但是，目前绿光如何通过信号转导机制调控气孔运动还存在知识空白。

值得注意的是，有研究表明气孔对于光照的响应在早期的维管植物中可能缺失 [42-43]。例如，在蕨类薄囊蕨纲 (Leptosporangiopsida) 和水龙骨纲 (Polypodiopsida) 中，其气孔对蓝光无响应 [42-43]；而在其他一些蕨类或裸子植物如苏铁 (*Cycas revoluta*) 中，其气孔对红光无响应，仅在蓝光存在时开放，这些差异有可能是由进化过程中选择压力与生长光环境的不同所致 [42]。

1.2 水分

气孔的一个重要生理功能是防止植物体水分过度流失，因此气孔对水分胁迫 (包括土壤水分亏缺和空气湿度降低) 十分敏感 [44-49]。植物在受到水分胁迫时，会产生大量 ABA，因此气孔对水分胁迫响应的研究主要集中在保卫细胞响应 ABA 的信号转导过程 [7,50]。目前已知 ABA 信号的主要转导途径包括受体 PYR1/PYL/RCAR 蛋白、蛋白磷酸酶 PP2Cs、蛋白激酶 SnRK2s，以及 S (slow) 与 R (rapid) 型阴离子通道。具体过程为：PYR/PYL/RCAR 受体在 ABA 存在的情况下结合 PP2C 蛋白磷酸酶 [51-52]，导致保卫细胞中的 SnRK2s 激酶 OST1 (open stomata 1) 活化，OST1 再通过激活质膜上的 S 与 R 型阴离子通道，使阴离子外流，致使质膜去极化，从而激活 K^+ 外流通道。各种离子的外流引起保卫细胞失水，使保卫细胞液泡膨压降低、体积减小，最终导致气孔孔隙的关闭 [53-57]。除此以外，还有研究表明 U-box 泛素连接酶、GHR1 和 EAR1 蛋白等重要组分都参与了保卫细胞对 ABA 响应的调控 [58-60]。

早期的研究者认为 ABA 在根部产生并在土壤水分亏缺时向上运输从而关闭气孔 [61]，之后的嫁接实验表明即使根部无法合成 ABA，土壤水分亏缺也可以引起气孔关闭 [62]，这说明可能由根部产生的某种信号诱导了地上部分 ABA 的合成 [62-63]。近年的一项重要研究表明多肽 CLE25 (clavata3/embryo-surrounding region-related 25) 可以感知土壤水分亏缺，并作为从植物根部运输到叶片的远程信号，诱导叶片中 ABA 的积累，引起气孔关闭 [44,64]。虽然该信号传递速度较慢，但可能是植物感知自然条件下土壤逐渐干燥的一种重要调控机制 [64-65]。

除对土壤水分亏缺的感知以外，气孔也可以感知因外界空气湿度降低导致的饱和水气压亏缺 (vapor pressure deficit,VPD) 的增加，但关于 ABA 是否参与了 VPD 诱导的气孔关闭仍存在较大争议 [66]。研究者发现，将三种不同的被子植物叶片置于 VPD 加倍的环境条件下，叶片 ABA 合成相关基因明显上调，ABA 含量增加，气孔趋于关闭 [67-69]；而在不能正常合成 ABA 的突变体中，气孔则会缺乏对 VPD 上升的响应 [70]。但是，也有研究表明 ABA 合成或感知缺陷突变体的气孔在高 VPD 下会像野生型一样关闭 [71-72]。近年来的一项研究表明，在高 VPD 下，ABA 受体的六重突变体 *pyr1/pyl1/pyl2/pyl4/pyl5/pyl8* 的气孔还是会趋于关闭，但是比野生型气孔关闭所需时间长，说明 ABA 信号转导并不是唯一参与高 VPD 下气孔关闭过程的机制 [73]。还有研究表明，ABA 信号转导途径中的重要蛋白激酶 OST1 包含 ABA-box (与 ABA 结合从而激活 OST1) 和 SnRK2- box (不结合 ABA，但在高 VPD 下激活) 两个调控域 [74-75]。OST1 激酶缺乏 ABA-box 的突变体不能响应 ABA，但其气孔在高 VPD 下仍然趋于关闭 [75]。该结果表明 OST1 激酶可能位于 ABA 与非 ABA 响应的交互点，其可以在不依赖 ABA 的条件下响应高 VPD[66,73,75-76]。

上述水分胁迫引起的气孔关闭可被视为保卫细胞的主动调节。除此以外，在一些早期维管植物中，水分胁迫会直接引起保卫细胞内的水分减少，导致气孔被动关闭 [44,46,48,77]。后续的研究发现，有些蕨类植物的气孔也可以受到 ABA 的调节 [78-79]。因此，早期维管植物可能根据物种和环境条件的不同，而使用被动和主动气孔调节策略应对水分胁迫 [78,80]，但在被子植物和多数裸子植物中，气孔关闭主要是主动调节的结果 [1,48,66-67,78,81-82]。

1.3 CO_2浓度

CO_2 不仅是光合作用所必需的底物，同时也是影响气孔运动的重要因子。无论是离体表皮上的气孔还是单独的保卫细胞原生质体都能够对 CO_2 浓度的变化做出响应，表明保卫细胞中也存在独立的 CO_2 感知和信号转导通路[83-86]。目前的研究表明，CO_2 首先通过跨膜扩散或 PIP2 (plasma membrane intrinsic protein 2) 水通道蛋白 (aquaporin) 两种方式进入保卫细胞[87-88]，之后在碳酸酐酶 βCA1 和 βCA4 (β-carbonic anhydrase 1 and 4) 作用下催化为 HCO_3^-，HCO_3^- 可以作为第二信使介导气孔对 CO_2 的响应[89-90]。最新的研究表明，保卫细胞的 CO_2 传感器可能是由促分裂原活化蛋白激酶 MPK4 和 MPK12 (mitogen activated protein kinase 4 and 12) 以及 HT1 (high leaf temperature 1) 蛋白共同组成[91-97]。当保卫细胞内 HCO_3^- 浓度低时，HT1 磷酸化并激活 CBC1 (convergence of blue light and CO_2) 蛋白激酶[98-99]，引起气孔开放；而当保卫细胞内 HCO_3^- 浓度高时，积累的 HCO_3^- 可以触发 MPK4/12 与 HT1 的结合，从而抑制 HT1 激酶活性，导致 CBC1 激酶活性下调，最终引起气孔关闭[97]。另外，胞内 HCO_3^- 还可以直接激活 S 型阴离子通道[89,91]，使得 Cl^- 和 NO_3^- 流出保卫细胞，促进气孔关闭过程[100-101]。这一过程可调节约 20% 的 S 型阴离子通道活性[102]。

除此以外，ABA 信号转导途径也可以增强气孔对 CO_2 升高时的关闭响应[57,103]。近年来的一项研究表明，在高 CO_2 诱导时，ABA 受体的六重突变体 *pyr1 pyl1 pyl2 pyl4 pyl5 pyl8* 和 OST1 激酶突变体 *ost1-3* 的气孔关闭速度减缓[57]，说明保卫细胞内的 ABA 转导途径与 OST1 激酶活性可以促进气孔对高 CO_2 的响应。

1.4 温度

气孔开闭还会受到温度信号的精细调控，以平衡水分流失与维持叶温的关系。早期的研究者发现，在不含 CO_2 的黑暗环境中，高温 (>35 °C) 可以诱导蚕豆 (*Vicia faba*) 离体表皮上的气孔打开，并且这种响应与保卫细胞内 K^+ 的积累有关[104-105]。对保卫细胞原生质体的进一步研究表明，当温度从 13 °C 升至 20 °C 时，膜上 K^+ 外流与内流流量均增加，而当温度继续升高 (从 20 °C 升至 28 °C)，K^+ 外流流量降低而内流流量继续增加，即在较高温度下 K^+ 内流与外流对温度响应的差异导致了气孔开放[106]。这些研究表明保卫细胞可能具有独立感知温度变化的相关分子机制。后续的研究将叶片置于黑暗中，并使用不同温度进行处理，发现气孔开度会随温度的增加而增加；而且将高温处理后的叶片重新放置到低温环境中，气孔开度会减小，表明气孔对温度变化的响应是双向且可逆的[107]。尽管光照 (辐射) 可能会引起温度的变化，以及温度也会伴随 VPD 的变化，但在控制光照与 VPD 的条件下，在升高温度后可以观察到多个物种的更大的 g_s[108-109]。近年来的研究表明，高温刺激 (38 °C) 引起的气孔开放与呼吸爆发氧化酶同源蛋白 D (respiratory burst oxidase homolog protein D, RBOHD) 介导的活性氧产生有关，并且可以将气孔开放的信号传递给未经高温刺激的叶片[110]。最近在对玉米 (*Zea mays*) 的研究中发现，MPK20 蛋白激酶突变体的气孔在高温下开度更大，而其过表达株系的气孔开度则更小，因此 MPK20 可能是高温诱导的气孔开放信号转导途径中的一个重要负调控因子[111]。除此以外，近年来的研究也发现高温下的气孔开放与气孔的蓝光响应途径 (见第 1.1 章节) 存在交互性，其中向光素 PHOT1/2 蛋白、BLUS1 蛋白激酶以及质膜 H^+-ATPase 都有可能参与了高温下气孔开放的调控[112]。综上所述，在高温诱导气孔开放的过程中存在多种机制与多个可能的高温信号整合位点[112]，需要更多的研究来找到这些信号整合位点并阐明气孔温度响应的机制。

在过去的几十年中，植物气孔运动响应外界环境变化的调控机制受到了广泛关注。但由于篇幅有限，本文难以阐述详尽，未在正文中提及的其他参与信号转导途径的重要组分可参见表 1。

2 气孔发育的调控

在短期内，植物主要通过上述各种机制调节气孔运动对环境变化做出响应；但在长时间尺度上，植物还可以通过对于气孔发育的调控适应外界生长环境[121–124]。

在具有肾形保卫细胞气孔的模式植物拟南芥中，幼叶表皮最初由未分化的原表皮层细胞 (protoderm) 构成，随着发育过程的进行，原表皮层细胞最终分化为叶片表皮中的三种细胞类型：表皮毛、表皮细胞和保卫细胞[125]。气孔发育的第一步是原表皮层细胞转变为拟分生组织母细胞 (meristemoid mother cell, MMC)，然后不对称分裂形成一个较大的气孔谱系基细胞 (stomatal lineage ground cell, SLGC) 和一个较小的拟分生组织细胞 (meristemoid cell, MC)。其中 SLGC 可以分化为表

表1 其他参与气孔运动响应外界环境变化的信号转导途径组分

环境因素		气孔运动响应	信号转导途径中的其他重要组分	参考文献
光照	蓝光	开放	CRY1/CRY2 (cryptochrome 1/2)	[113]
			CBC1/ CBC2 (convergence of blue and CO_2 1/2)	[98]
	红光	开放	RopGEF2-ROP7/2 (ROPs-specific guanine nucleotide exchange factors 2 – ROP7/2)	[114]
			MKK2-MPK2 (mitogen-activated protein kinase kinase 2 - mitogen-activated protein kinase 2)	[115]
水分缺失/VPD升高		关闭	参与ABA调控途径的其他组分*	[55,116-117]
CO_2浓度增加		关闭	RHC1 (resistant to high CO_2)	[118]
			AT2G40180	[119]
温度增加		开放	PME34 (pectin methyl esterase 34)	[120]

* 由于本部分内容较多，在本表格中就不再逐一列举，具体请参考所列出的相关文献。

皮细胞或者进行间隔分裂 (spacing division)，形成一个新的 MC 并与原来的 MC 隔开。而 MC 可以直接分化为保卫细胞母细胞 (guard-cell mother cell, GMC)，后经一次对称分裂 (symmetric division) 形成气孔；或进行一到三次的向内螺旋式不对称增殖分裂 (amplifying division) 形成新的 SLGC 和被 SLGC 包围的晚期 MC 细胞，然后晚期 MC 细胞分化为 GMC，之后再经一次对称分裂形成气孔 [125-128]。

气孔发育过程严格遵循单细胞间隔的模式，即气孔间不会直接相互接触，两个气孔间至少存在一个非气孔表皮细胞，以确保气孔的适当开合 [122]。其中 EPF1/2 (epidermal patterning factor 1/2) 小肽负调控气孔密度，其通过调节气孔前体细胞的分裂模式来抑制气孔的形成 [129-133]。产生于叶肉组织的另一种小肽 EPFL9 (或称 STOMAGEN) 则正调控气孔密度 [134-135]，小肽的受体蛋白 ER (erecta) 和 ERL (erecta-like) 也正调控气孔密度，它们的相互作用在调控气孔发育过程中起作用 [128,136]。此外，SDD1 (stomatal density and distribution 1) 蛋白酶也是气孔发育过程中的负调控因子，但其发挥作用的信号途径至今仍不清楚 [127,137-138]。

上述气孔的发育过程还受到 bHLH (basic helix-loop-helix) 家族转录因子 SPCH (SPEECHLESS)、MUTE、FAMA，以及结合蛋白 SCREAM (SCRM/ICE1) 和 SCRM2 的调控，缺失这些转录因子将导致气孔无法正常形成 [125,129,139]。虽然哑铃形保卫细胞气孔的发育过程与肾形保卫细胞气孔发育过程有诸多不同且尚未完全明确 [4,125,129]，但其均受到这些 bHLH 家族转录因子的调控 [125,129,139]。

近年来越来越多的研究将环境信号整合到气孔发育的通路中，从而探讨气孔发育对于环境的响应机制。

(1) 光强增加一般会促进气孔的形成，高光强下生长的植株叶片的气孔指数增加 [140-145]。研究表明，植物中除向光素外的另一种蓝光受体隐花色素 (cryptochrome, Cry) 与远红外 / 红光光受体光敏色素 Phy A/B 共同介导了光信号对气孔发育的调节，表现为突变体植株在相应光谱下生长时，比野生型具有更低的气孔指数与密度 [141,143,145]。在 Cry 和 Phy 信号网络下游的组分中，COP1 (constitutive photomorphogenesis 1)、SPA1 (suppressor of phytochrome A1)、PIFs、B-GATA 之间的相互作用能够将光信号传递给调控气孔形成的转录因子 SPCH、MUTE 和 FAMA，从而实现对气孔发育的调控 [128,143]。

(2) 水分状况也会影响气孔发育，但目前对其具体分子机制知之甚少。在多个物种中观察到空气湿度过低、土壤水分亏缺或渗透胁迫时，叶片的气孔密度降低 [146-154]。其中，ABA 的积累被认为是抑制气孔发育的重要因素之一 [155]；也有研究表明，渗透胁迫发生时，MAPK 级联反应介导的磷酸化使得 SPCH 蛋白水平降低，从而抑制表皮上气孔的发育 [148,156]。另外，湿度较低导致的气孔密度下降可以通过其 DNA 甲基化进行代际遗传 [157-158]。这表明植物可以通过调控气孔发育来精确调节水分利用效率以优化生长，但这一过程可能取决于具体的物种与环境 [77]。

(3) 随着大气 CO_2 浓度的升高，气孔密度一般会下降，但在一些物种中也存在 CO_2 浓度升高气孔密度增加的现象 [124,159]。对 1787—1987 (大气 CO_2 浓度：280~340 ppm) 年的植物标本进行分析并结合控制实验的研究表明，CO_2 浓度上升会导致多数植物的气孔密度下降 [160]，并且这一结果也得到了很

多后续研究的支持[124,159,161]。之后，针对模式植物拟南芥的研究鉴定了首个在高 CO_2 下负调控气孔发育的基因 *HIC* (*high carbon dioxide*)，在 CO_2 浓度倍增的条件下，该突变体的气孔密度比野生型高42%[162]。研究表明，*HIC* 基因编码一个参与角质层合成的长链脂肪酸合成酶[162]，其可能通过改变角质层成分影响叶片的 CO_2 进入和光吸收，从而间接调控气孔发育，但目前对于 HIC 影响气孔发育的具体途径仍不清楚[127-128,163-164]。之后还有研究表明，碳酸酐酶 βCA1 和 βCA1 突变体 *ca1ca4* 的气孔密度在高 CO_2 时显著增加[89]，这主要是由于在突变体中气孔发育的负调控因子 EPF2 小肽的表达量显著降低[165]，说明在气孔运动响应 CO_2 途径中发挥作用的碳酸酐酶也参与了气孔发育的调控。通过联合蛋白质组学的分析结果发现，高 CO_2 下诱导的胞外 CRSP (CO_2 response secreted protease) 可通过切割 EPF2 前体肽，激活 EPF2，抑制气孔发育[165]。因此，高浓度 CO_2 抑制气孔发育的机制可能主要由 CRSP 与 EPF2 所介导[165]。除对 CO_2 浓度的直接响应外，子代植物的气孔发育还可能受到母代植物所生长环境的 CO_2 影响，即代际遗传的影响。最近的研究表明拟南芥和水稻 (*Oryza sativa*) 在高 CO_2 环境下经多代种植后，子代植株表现出气孔间距增加或气孔密度降低[166-168]，表明代际间的表观遗传调控可能参与气孔发育对高 CO_2 的适应[167]。

(4) 近年来的研究表明，高温可以通过 PIF4 抑制 SPCH 启动子的活性和表达，导致气孔密度和指数降低[169-170]，而 *pif4* 突变体则会在高温中仍保持较高的气孔指数与密度[170]。SPCH 也可以反过来直接结合并抑制 PIF4 的转录，表明 PIF4 与 SPCH 形成负反馈调控，这有利于植物在应对温度波动时通过调控气孔发育改善生长状况[170]。需要注意的是，高温会促进气孔张开 (见前文 1.4 章节)，但会抑制气孔发育。这可能是由于在自然情况下，高温通常伴随着水分胁迫，因此植物必须在增加叶片蒸腾冷却的同时防止水分过度流失。气孔运动与气孔发育的“矛盾”表型可能反映了植物在高温条件下的复杂权衡机制。

3 叶片上下两面气孔的差异

在前述关于气孔运动与气孔发育的研究中，一般仅使用下表面的气孔开度和气孔密度来衡量气孔对外界环境的响应[121]，这是因为在多数植物尤其是木本植物中，气孔仅分布于叶片的下表面，且下表面表皮较易剥离，便于气孔观察。但是对于很多草本植物 (包括常用模式植物拟南芥) 来说，气孔在上下表面均有分布[171-172]。两面气孔的分布方式能够缩短气体由气孔下腔到叶绿体的距离，促进 CO_2 快速扩散，从而使光合速率达到最优[173-176]。对于叶片上下两面气孔对环境响应差异的研究将加深对于气孔如何调节 CO_2 向叶内扩散的理解，是气孔与光合作用相关研究的重要内容。

在自然环境中，叶片上下表面的光环境不同。由于光照方向主要为自上而下，叶片上表面主要接收直射光，下表面则主要接收透射光和周围环境的反射光。具体而言，照射在叶片上表面的光，光强较强、能量较高、光谱较完整；而光穿过叶片后，大量红蓝光被叶肉细胞光合作用所吸收利用，因此能够到达叶片下表面的光，光强较弱、能量较低，且以绿光为主[31,35,171]。对蚕豆[177-178]、向日葵[40-41]、陆地棉 (*Gossypium hirsutum*)[179]、鸭跖草 (*Commelina communis*)[180-181]、苍耳 (*Xanthium strumarium*)[182] 等草本植物的研究均表明，两面气孔的光响应存在差异。其中，通过生理实验发现，当对向日葵叶片两面分别照光，并保持叶内 CO_2 浓度在叶片两侧一致时，在所有光照强度下，叶片下表面的 g_s 均高于上表面[40-41]。此外，绿光可以诱导向日葵下表面气孔开放，但不能诱导上表面气孔开放，这些光响应的差异并不能够通过翻转叶片使生长光环境改变而消除[41]。在调控机制方面，Goh 等[177-178] 发现上下表面保卫细胞的质膜 H^+- ATPase 泵送 H^+ 的能力相似，但上表面保卫细胞需要更多的光子来激活质膜 H^+-ATPase。

在气孔发育方面，主要的研究集中在对不同植物物种叶片上下表面气孔密度的统计分析。Muir[183] 为表征气孔的分布方式，将叶片上表面气孔密度与总气孔密度之比定义为气孔比率 (stomatal ratio, SR)。由其定义可知，当 SR=0 时，叶片仅下表面具有气孔，即为气孔下生叶；当 SR=1 时，叶片仅上表面具有气孔，即为气孔上生叶；当 $0 < SR < 1$ 时，叶片具两面气孔[183]。研究表明，不同植物的 SR 差异很大，但在某些物种中 SR 表现出高度可塑性，随生长环境的变化而变化。例如，随着光照强度的降低，蓝桉 (*Eucalyptus globulus* ssp. *globulus*) 成龄叶片上表面气孔密度减小，SR 降低[184]。多数物种在下表面的气孔密度较上表面更高[172,185-186]，但是在禾本科、莎草科等具有直立叶片的植物中，上下表面的气孔密度通常比较接近[186]。这些研究

说明气孔发育在叶片的上下表面可能受到不同的调控[187]，但调控机制尚未明确。

4 叶肉光合作用对气孔运动的调控

气孔是CO_2进入叶片内部的重要通道，因此气孔运动对于叶片的光合作用，特别是叶肉细胞的光合作用至关重要。在较为稳定的环境下，气孔对光照和CO_2变化的响应速度比光合速率至少慢一个数量级。在水分充足的情况下，叶片光合速率在光强变化的几秒内就发生改变，但g_s发生改变则需几分钟到几十分钟，气孔响应的滞后导致较低的g_s，限制CO_2经气孔向叶片内部的扩散，从而限制光合作用速率[188-189]。有研究表明，当光强增加时，由于气孔响应的滞后，可使光合速率减少约20%，此外g_s的下降也会影响叶片蒸腾散热过程，从而影响叶表温度，这可能对植物的光合速率和生长产生进一步的影响[31,190]。当水分胁迫发生时，g_s减小导致的叶温升高将影响光合作用的正常进行，导致光系统II的最大量子产额(Fv/Fm)和Rubisco酶活性降低[191-192]。这些气孔运动对光合作用产生的限制被称为“光合作用的气孔限制”[193-195]。

另一方面，Wong等[196]通过研究不同植物在不同光照条件下的光合速率和g_s的关系，发现这二者存在良好的线性相关，提出了气孔运动与光合作用可能存在“相协调”(coordination)的关系，即叶肉光合也可能通过某种机制调节气孔的运动。之后有研究通过控制叶片上下表面的CO_2浓度，证明了叶片g_s与叶内CO_2浓度(C_i)而不是环境CO_2浓度(C_a)的变化相关[197]。通常认为是光合作用发生时C_i的变化介导了光合作用对气孔运动的调节，因为C_i是由经气孔进入叶片内部的CO_2和光合作用所消耗叶片内部CO_2的量共同决定，即在光照增加时，光合作用的进行导致了叶片内部CO_2被消耗，气孔开放以响应C_i的降低；反之当光合作用速率因其他环境因子减弱时，此时较高的C_i则导致气孔关闭[197-198]。然而，随后的一些研究表明即使保证C_i恒定，当红光光强发生变化时g_s也会发生变化[28]。此外，在利用反义RNA技术抑制卡尔文-本森-巴萨姆循环中关键酶表达的转基因植株中，即使C_i一直较高，气孔也会响应光强增加而打开[199-200]。这些结果说明C_i无法完全解释叶肉光合作用对气孔运动的调节[31,201–203]，保卫细胞对红光的响应还可能包含其对某种“叶肉信号”的响应。

存在“叶肉信号”这一想法最早可以追溯到1954年，Heath和Russell[204]认为气孔运动可能受到叶肉向表皮传递的间接化学或电信号的影响。后来的研究推论叶肉信号可能是一种光合作用的代谢物，其能够平衡光合作用的电子传递和Rubisco酶的限制[28,196]。Lee和Bowling[205-207]在1992年首次通过实验证实叶肉信号的存在，他们发现将离体的表皮或保卫细胞悬浮在从光照后叶片中分离的叶肉细胞或叶绿体悬浮液中，气孔会恢复对光的响应。通过比较完整叶片与离体表皮上的气孔对光的响应，发现离体表皮上的气孔对光照的响应缺失或降低，从而证明叶肉在控制气孔光响应中的作用[198,205-206,208-211]。另外，经过研究发现，将离体的表皮放置到不同物种叶肉上，气孔也可以恢复对光的响应，表明叶肉向表皮传递的信号在不同物种间可能是通用的[209-216]。在后续的研究中对于信号物质是气态还是液态产生了争论。Sibbernsen和Mott[214]发现当叶片细胞间隙充满液体时，气孔关闭，他们认为是液体的存在阻隔了某种气态信号的传递；而在离体的表皮下方放置一电极，通过施加正负电压产生不同的气相离子时，气孔会有所响应[213-214]。在这之后，Fujita等[210-211]通过在离体表皮与叶肉之间插入玻璃纸(只能液体透过)或聚乙烯薄膜(液体和气体均不能透过)，证明叶肉信号是水溶性的，并认为其可能通过质外体途径从叶肉组织向保卫细胞传递。目前的研究已经表明蔗糖[217-218]、苹果酸[219-221]等有可能充当液态信号介导叶肉光合对气孔运动的调控，但也有理论主张电子传递链氧化还原状态也能介导叶肉光合作用对气孔运动的调控[27]。最近，Głowacka等[222]通过改变光系统II亚基S的表达量控制电子传递链的氧化还原状态验证了这一理论的可能性。

上述这些结果都表明叶肉的光合作用能够调控气孔的运动，但具体的调控机制还有待进一步阐明。

5 不同光合代谢途径植物在气孔运动与发育方面的差异

目前大多数有关气孔的研究主要集中于C_3植物，这可能是因为C_3植物种类较多且其气孔更易进行观察与研究[188]。除C_3植物以外，根据光合代谢途径划分，还存在C_4植物与CAM(crassulacean acid metabolism)植物。本节将简要介绍C_4以及CAM植物气孔运动与发育的研究成果。

C_4植物虽然仅占植物物种总量的3%，但是其总初级生产力可占全球植物的23%左右[223-224]。

C_4 植物的高生产力得益于其高效的光合作用过程，特别是空间隔离的磷酸烯醇丙酮酸羧化酶 (phosphoenolpyruvate carboxylase, PEPCase) 介导的 CO_2 富集过程，可使得 Rubisco 酶周围的 CO_2 浓度提高，从而有效抑制光呼吸的发生 [224-225]。如前所述，光合作用速率与气孔导度存在“相协调”的关系 (见第 4 章节)。C_3 植物气孔导度降低后，光合作用速率也随之下降，使得内在水分利用效率 (intrinsic water-use efficiency，即光合作用速率与气孔导度的比值) 不发生显著变化 [226]；而 Cano 等 [227] 发现，在所有 C_4 植物亚型中，植物的内在水分利用效率均同气孔导度呈负相关，但与光合作用速率没有呈现显著的相关关系。此外，有研究表明 C_4 植物气孔对 C_i 变化的敏感性大于 C_3 物种 [203,228-230]，当 CO_2 浓度增加时，C_4 植物相比于 C_3 植物更容易关闭气孔 [231]。在气孔的光响应研究中也发现，无论 C_3 与 C_4 植物间亲缘关系远近，C_4 植物气孔对蓝光的敏感性比较低 [232-233]。

除了气孔运动的差异，也有研究表明 C_4 植物和 C_3 植物之间具有气孔大小与气孔密度的差异 [234]。最近的一项研究表明在 C_3 向 C_4 演化的过程中，叶片气孔密度趋向降低，而保卫细胞尺寸则趋向于增大，这导致了最大气孔导度的降低，随着向 C_4 途径的演化，气孔发育正调控的小肽蛋白 STOMAGEN (或称 EPFL9) 在植物中表达水平也降低 [235]。但是目前关于在 C_3 向 C_4 演化过程中，气孔发育如何变化仍存在较多未知 [233]。

CAM 植物气孔通常在夜晚张开，CO_2 进入叶片内部，在 PEPCase 催化下生成草酰乙酸后被还原为苹果酸等四碳化合物并储存在液泡中，此时 C_i 降低；而在光照期间，气孔通常关闭，液泡内积累的四碳化合物脱羧释放 CO_2，C_i 升高，以供 Rubisco 酶固定 [236]。CAM 植物的气孔开闭模式一直以来被认为是其响应 C_i 变化的结果 [236-238]。然而后续的一项研究表明，在景天科 *Kalanchoe* 属植物中，其气孔导度对 C_i 的变化无响应，表明 C_i 并不是影响 CAM 植物气孔开闭的唯一因素 [239]。通过研究发现 C_3 与 CAM 途径间可进行转换的植物，如刺戟木科的 *Portulacaria afra* 和番杏科的 *Mesembryanthemum crystallinum*，当它们使用 C_3 途径进行光合碳同化时，蓝光和红光照射可促进其气孔开放，而当其转变为 CAM 途径时，光照诱导的气孔开放效应消失，气孔显现出一定程度的关闭 [240-241]。类似地，对于天门冬科植物 *Agave americana* 的转录组分析结果也表明，CAM 植物的气孔可能不具备对蓝光的响应 [242]。在其他 CAM 植物中，如凤梨科植物 *Tillandsia recurvata* 的气孔能够在夜间对空气湿度的变化做出响应 [243]。综上所述，在不同的 CAM 植物中，可能存在着多种与 C_3 植物不同的气孔调控机制 [238,242]。

6 优化植物气孔性状，协同提升作物水分利用效率与产量

随着气孔运动及气孔发育的调控机制被解析，如何利用这些理论实现当前和未来气候变化条件下植株水分利用效率和生物量或产量协同提升是当前研究的热点。近十年的研究成果主要集中于通过调控气孔发育、气孔对光的响应，以及保卫细胞糖代谢三个方面来提高光合速率、作物产量，或优化水分利用效率 [192,244]。

在改变气孔发育方面，通过超表达分泌性小肽的基因 *EPF1* 和 *EPF2*，可以有效降低拟南芥的气孔密度 [130,132-133]，显著提高植株的抗旱能力和水分利用效率，同时不影响 (或轻微降低) 植株的光合速率 [131,245-246]。该成果已在水稻 [247]、大麦 (*Hordeum vulgare*)[246]、番茄 (*Solanum lycopersicum*)[248]、杨树 [*Populus nigra* × (*Populus deltoides Populus nigra*)][249] 中得到了广泛的应用。与之相似，超表达另一种气孔发育负调控因子 SDD1，有效地降低了玉米和番茄植株的气孔密度，使其水分利用效率和抗旱性均得到了显著提高 [250-251]。这些成果表明，在未来气候变化条件下，降低气孔密度可能是提高植株水分利用效率和抗旱能力的可行策略 [129,252]。与之相反，超表达气孔发育正调控小肽基因 *EPFL9* 的植株和 *epf1epf2* 突变体的气孔密度增加，虽然叶片 g_s 和光合速率也有所增加，但是由于其蒸腾失水过多，水分利用效率显著下降，导致在一般生长条件下植株的生物量并未明显增加 [131,155]。今后可以考虑在水分充足的条件下，比如在条件可控的植物工厂等设施农业中，应用增加气孔密度技术，通过提升 g_s 和光合速率，实现生物量或产量的提高 [192]。

在改变气孔对光响应方面，超表达光信号转导途径中的质膜 H^+-ATPase，可以有效提高拟南芥植株在光照条件下的气孔开度、气孔导度和光合速率 [253]。虽然超表达株系的水分利用效率有所下降，但是植株的抗旱性并未受到影响，因此在强光下生长的植株的生物量和角果数量显著增加 [253]。之后的研究表明，通过在水稻中利用组成型启动子超表达质膜 H^+-ATPase，同时促进了水稻植株叶片的

光合能力与根部对于铵的吸收能力，使超表达株系可以在不改变水分利用效率的情况下，显著提升产量和氮利用效率[254]。此外，调控气孔在光照条件下的开闭速度也是优化光合作用和水分利用效率的有效途径。Papanatsiou 等[255]使用合成生物学的方法，构造了光控 K^+ 通道 BLINK1，并在拟南芥保卫细胞中表达，使植株在光照下的气孔开放与黑暗下的气孔关闭速度显著提升。在波动光下，可以在不影响水分利用效率的同时，增加植株生物量的积累[255]。

此外，调控保卫细胞糖代谢基因的表达也可以有效地提高光合作用。在烟草 (*Nicotiana tabacum*) 保卫细胞中超表达蔗糖合酶 3 基因 *SUS3* (*sucrose synthase 3*) 或在水稻中敲除己糖激酶 1 基因 *HXK1* (*hexokinase 1*) 可增加 g_s、光合速率以及植物生物量或产量[256-257]。但目前尚不清楚保卫细胞糖代谢在调节气孔运动中的具体作用，其机制有待进一步研究。

7 展望

气孔作为 CO_2 进入叶片的最主要通道，是光合作用研究的重要内容。迄今为止，虽然在气孔研究方面取得了很多成果，但是气孔自身的调控机制、气孔与光合作用的相协调，以及通过优化气孔提升植物光合与生物量方面还存在许多亟待解决的问题。

在气孔运动调控机制方面：(1) 质膜 H^+-ATPase 的激酶一直未能找到，这是气孔保卫细胞光响应信号转导中的核心问题；(2) 除蓝光和 ABA 有明确的受体蛋白以外，气孔保卫细胞感受其他光质、空气湿度、CO_2、温度的受体都尚未明确或存在一定的争议；(3) 气孔运动响应两种及以上环境因素的机制、哑铃形保卫细胞气孔运动的调控机制还存在许多研究空白。在气孔发育调控机制方面：(1) 气孔发育如何响应外界环境因素变化还有许多不明点；(2) 哑铃形保卫细胞气孔的发育过程尚未完全明确；(3) 气孔大小的调控机制一直未明。另外，前文中提及的叶片上下表面气孔差异、叶肉光合对气孔运动的调控、不同光合途径植物的气孔差异，这三方面都是理解气孔与光合作用相协调的关键问题，是今后需要重点开展的理论研究内容。在这些理论研究的基础上，如何进一步实现通过精准调控气孔协同提升植物水分利用效率、生物量或产量也是亟待解决的课题。这些课题能够为全球的粮食生产和陆地生态系统碳汇能力的提升提供重要的解决方案。

[参 考 文 献]

[1] Brodribb TJ, McAdam SAM. Passive origins of stomatal control in vascular plants. Science, 2011, 331: 582-5

[2] Raven JA. Selection pressures on stomatal evolution. New Phytol, 2002, 153: 371-86

[3] Lawson T, Matthews J. Guard cell metabolism and stomatal function. Annu Rev Plant Biol, 2020, 71: 273-302

[4] Hepworth C, Caine RS, Harrison EL, et al. Stomatal development: focusing on the grasses. Curr Opin Plant Biol, 2018, 41: 1-7

[5] McKown KH, Bergmann DC. Grass stomata. Curr Biol, 2018, 28: R814-6

[6] Murata Y, Mori IC, Munemasa S. Diverse stomatal signaling and the signal integration mechanism. Annu Rev Plant Biol, 2015, 66: 369-92

[7] Schroeder JI, Allen GJ, Hugouvieux V, et al. Guard cell signal transduction. Annu Rev Plant Physiol Plant Mol Biol, 2001, 52: 627-58

[8] Kim TH, Böhmer M, Hu H, et al. Guard cell signal transduction network: advances in understanding abscisic acid, CO_2, and Ca^{2+} signaling. Annu Rev Plant Biol, 2010, 61: 561-91

[9] Darwin F. IX. Observations on stomata. Philos Trans R Soc Lond Ser B Contain Pap Biol Character, 1898, 190: 531-621

[10] Sharkey TD, Raschke K. Effect of light quality on stomatal opening in leaves of *Xanthium strumarium* L. Plant Physiol, 1981, 68: 1170-4

[11] Zeiger E, Hepler PK. Light and stomatal function: blue light stimulates swelling of guard cell protoplasts. Science, 1977, 196: 887-9

[12] Zeiger E, Talbott LD, Frechilla S, et al. The guard cell chloroplast: a perspective for the twenty-first century. New Phytol, 2002, 153: 415-24

[13] Kinoshita T, Doi M, Suetsugu N, et al. phot1 and phot2 mediate blue light regulation of stomatal opening. Nature, 2001, 414: 656-60

[14] Christie JM. Phototropin blue-light receptors. Annu Rev Plant Biol, 2007, 58: 21-45

[15] Shimazaki K, Doi M, Assmann SM, et al. Light regulation of stomatal movement. Annu Rev Plant Biol, 2007, 58: 219-47

[16] Inoue S, Kinoshita T. Blue light regulation of stomatal opening and the plasma membrane H^+-ATPase. Plant Physiol, 2017, 174: 531-8

[17] Marten H, Hedrich R, Roelfsema MRG. Blue light inhibits guard cell plasma membrane anion channels in a phototropin-dependent manner. Plant J, 2007, 50: 29-39

[18] Andrés Z, Pérez-Hormaeche J, Leidi EO, et al. Control of vacuolar dynamics and regulation of stomatal aperture by tonoplast potassium uptake. Proc Natl Acad Sci USA, 2014, 111: E1806-14

[19] De Angeli A, Zhang J, Meyer S, et al. AtALMT9 is a malate-activated vacuolar chloride channel required for stomatal opening in *Arabidopsis*. Nat Commun, 2013, 4: 1804

[20] Takemiya A, Sugiyama N, Fujimoto H, et al. Phosphorylation of BLUS1 kinase by phototropins is a primary step in stomatal opening. Nat Commun, 2013, 4: 2094
[21] Takemiya A, Kinoshita T, Asanuma M, et al. Protein phosphatase 1 positively regulates stomatal opening in response to blue light in *Vicia faba*. Proc Natl Acad Sci USA, 2006, 103: 13549-54
[22] Takemiya A, Shimazaki K. *Arabidopsis* phot1 and phot2 phosphorylate BLUS1 kinase with different efficiencies in stomatal opening. J Plant Res, 2016, 129: 167-74
[23] Takemiya A, Yamauchi S, Yano T, et al. Identification of a regulatory subunit of protein phosphatase 1 which mediates blue light signaling for stomatal opening. Plant Cell Physiol, 2013, 54: 24-35
[24] Matthews JSA, Vialet-Chabrand S, Lawson T. Role of blue and red light in stomatal dynamic behaviour. J Exp Bot, 2020, 71: 2253-69
[25] Hayashi M, Inoue S, Takahashi K, et al. Immunohistochemical detection of blue light-induced phosphorylation of the plasma membrane H^+-ATPase in stomatal guard cells. Plant Cell Physiol, 2011, 52: 1238-48
[26] Hayashi M, Inoue S, Ueno Y, et al. A Raf-like protein kinase BHP mediates blue light-dependent stomatal opening. Sci Rep, 2017, 7: 45586
[27] Busch FA. Opinion: the red-light response of stomatal movement is sensed by the redox state of the photosynthetic electron transport chain. Photosynth Res, 2014, 119: 131-40
[28] Messinger SM, Buckley TN, Mott KA. Evidence for involvement of photosynthetic processes in the stomatal response to CO_2. Plant Physiol, 2006, 140: 771-8
[29] Kuiper PJC. Dependence upon wavelength of stomatal movement in epidermal tissue of *Senecio odoris*. Plant Physiol, 1964, 39: 952-5
[30] Ando E, Kinoshita T. Fluence rate dependence of red light-induced phosphorylation of plasma membrane H^+-ATPase in stomatal guard cells. Plant Signal Behav, 2019, 14: 1561107
[31] Lawson T, Terashima I, Fujita T, et al. Coordination between photosynthesis and stomatal behavior[M]// Adams III WW, Terashima. The Leaf: A Platform for Performing Photosynthesis. Cham: Springer International Publishing, 2018: 141-61
[32] Zhu M, Geng S, Chakravorty D, et al. Metabolomics of red-light-induced stomatal opening in *Arabidopsis thaliana*: coupling with abscisic acid and jasmonic acid metabolism. Plant J, 2020, 101: 1331-48
[33] Gao Y, Jiang W, Dai Y, et al. A maize phytochrome-interacting factor 3 improves drought and salt stress tolerance in rice. Plant Mol Biol, 2015, 87: 413-28
[34] Gao Y, Wu M, Zhang M, et al. Roles of a maize phytochrome-interacting factors protein ZmPIF3 in regulation of drought stress responses by controlling stomatal closure in transgenic rice without yield penalty. Plant Mol Biol, 2018, 97: 311-23
[35] Gao Y, Wu M, Zhang M, et al. A maize phytochrome-interacting factors protein ZmPIF1 enhances drought tolerance by inducing stomatal closure and improves grain yield in *Oryza sativa*. Plant Biotechnol J, 2018, 16: 1375-87
[36] Li Q, Zhou L, Chen Y, et al. Phytochrome interacting factor regulates stomatal aperture by coordinating red light and abscisic acid. Plant Cell, 2022, 34: 4293-312
[37] Terashima I, Fujita T, Inoue T, et al. Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. Plant Cell Physiol, 2009, 50: 684-97
[38] Smith HL, McAusland L, Murchie EH. Don't ignore the green light: exploring diverse roles in plant processes. J Exp Bot, 2017, 68: 2099-110
[39] Aasamaa K, Aphalo PJ. Effect of vegetational shade and its components on stomatal responses to red, blue and green light in two deciduous tree species with different shade tolerance. Environ Exp Bot, 2016, 121: 94-101
[40] Wang Y, Noguchi K, Terashima I. Distinct light responses of the adaxial and abaxial stomata in intact leaves of *Helianthus annuus* L. Plant Cell Environ, 2008, 31: 1307-16
[41] Wang Y, Noguchi K, Terashima I. Photosynthesis-dependent and -independent responses of stomata to blue, red and green monochromatic light: differences between the normally oriented and inverted leaves of sunflower. Plant Cell Physiol, 2011, 52: 479-89
[42] Doi M, Kitagawa Y, Shimazaki K. Stomatal blue light response is present in early vascular plants. Plant Physiol, 2015, 169: 1205-13
[43] Doi M, Wada M, Shimazaki K. The fern *Adiantum capillus*-veneris lacks stomatal responses to blue light. Plant Cell Physiol, 2006, 47: 748-55
[44] Gupta A, Rico-Medina A, Caño-Delgado AI. The physiology of plant responses to drought. Science, 2020, 368: 266-9
[45] Schulze ED, Turner NC, Gollan T, et al. Stomatal responses to air humidity and to soil drought[M]// Zeiger E, Farquhar GD, Cowan IR. Stomatal Function. Stanford: Stanford University, 1987 : 311-21
[46] Arve LE, Torre SL, Olsen JE, et al. Stomatal responses to drought stress and air humidity[M]// Shanker A, Venkates Warlu B. Abiotic Stress in Plants - Mechanisms and Adaptations. Rijeka: InTech, 2011
[47] Pantin F, Blatt MR. Stomatal response to humidity: blurring the boundary between active and passive movement. Plant Physiol, 2018, 176: 485-8
[48] Buckley TN. How do stomata respond to water status? New Phytol, 2019, 224: 21-36
[49] Tardieu F. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. J Exp Bot, 1998, 49: 419-32
[50] Hetherington AM. Guard cell signaling. Cell, 2001, 107: 711-4
[51] Ma Y, Szostkiewicz I, Korte A, et al. Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science, 2009, 324: 1064-8

[52] Park SY, Fung P, Nishimura N, et al. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science, 2009, 324: 1068-71
[53] Kuromori T, Seo M, Shinozaki K. ABA transport and plant water stress responses. Trends Plant Sci, 2018, 23: 513-22
[54] Munemasa S, Hauser F, Park J, et al. Mechanisms of abscisic acid-mediated control of stomatal aperture. Curr Opin Plant Biol, 2015, 28: 154-62
[55] Hsu PK, Dubeaux G, Takahashi Y, et al. Signaling mechanisms in abscisic acid-mediated stomatal closure. Plant J, 2021, 105: 307-21
[56] Imes D, Mumm P, Böhm J, et al. Open stomata 1 (OST1) kinase controls R-type anion channel QUAC 1 in *Arabidopsis* guard cells. Plant J, 2013, 74: 372-82
[57] Hsu PK, Takahashi Y, Munemasa S, et al. Abscisic acid-independent stomatal CO_2 signal transduction pathway and convergence of CO_2 and ABA signaling downstream of OST1 kinase. Proc Natl Acad Sci USA, 2018, 115: E9971-80
[58] Kong L, Cheng J, Zhu Y, et al. Degradation of the ABA co-receptor ABI1 by PUB12/13 U-box E3 ligases. Nat Commun, 2015, 6: 8630
[59] Wang K, He J, Zhao Y, et al. EAR1 negatively regulates ABA signaling by enhancing 2C protein phosphatase activity. Plant Cell, 2018, 30: 815-34
[60] Hua D, Wang C, He J, et al. A plasma membrane receptor kinase, GHR1, mediates abscisic acid- and hydrogen peroxide-regulated stomatal movement in *Arabidopsis*. Plant Cell, 2012, 24: 2546-61
[61] Zhang J, Schurr U, Davies WJ. Control of stomatal behaviour by abscisic acid which apparently originates in the roots. J Exp Bot, 1987, 38: 1174-81
[62] Holbrook NM, Shashidhar VR, James RA, et al. Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. J Exp Bot, 2002, 53: 1503-14
[63] Christmann A, Weiler EW, Steudle E, et al. A hydraulic signal in root-to-shoot signalling of water shortage. Plant J, 2007, 52: 167-74
[64] Takahashi F, Suzuki T, Osakabe Y, et al. A small peptide modulates stomatal control via abscisic acid in long-distance signalling. Nature, 2018, 556: 235-8
[65] Takahashi F, Kuromori T, Urano K, et al. Drought stress responses and resistance in plants: from cellular responses to long-distance intercellular communication. Front Plant Sci, 2020, 11: 556972
[66] Jalakas P, Takahashi Y, Waadt R, et al. Molecular mechanisms of stomatal closure in response to rising vapour pressure deficit. New Phytol, 2021, 232: 468-75
[67] McAdam SAM, Sussmilch FC, Brodribb TJ. Stomatal responses to vapour pressure deficit are regulated by high speed gene expression in angiosperms: rapid ABA biosynthesis during VPD transition. Plant Cell Environ, 2016, 39: 485-91
[68] Sussmilch FC, Brodribb TJ, McAdam SAM. Up-regulation of NCED3 and ABA biosynthesis occur within minutes of a decrease in leaf turgor but AHK1 is not required. J Exp Bot, 2017, 68: 2913-8
[69] Waadt R, Hitomi K, Nishimura N, et al. FRET-based reporters for the direct visualization of abscisic acid concentration changes and distribution in *Arabidopsis*. ELife, 2014, 3: e01739
[70] Cardoso AA, Brodribb TJ, Kane CN, et al. Osmotic adjustment and hormonal regulation of stomatal responses to vapour pressure deficit in sunflower. AoB Plants, 2020, 12: plaa025
[71] Assmann SM, Snyder JA, Lee YJ. ABA-deficient (*aba1*) and ABA-insensitive (*abi1-1*, *abi2-1*) mutants of *Arabidopsis* have a wild-type stomatal response to humidity. Plant Cell Environ, 2000, 23: 387-95
[72] Yaaran A, Negin B, Moshelion M. Role of guard-cell ABA in determining steady-state stomatal aperture and prompt vapor-pressure-deficit response. Plant Sci, 2019, 281: 31-40
[73] Merilo E, Yarmolinsky D, Jalakas P, et al. Stomatal VPD response: there is more to the story than ABA. Plant Physiol, 2018, 176: 851-64
[74] Belin C, De Franco PO, Bourbousse C, et al. Identification of features regulating OST1 kinase activity and OST1 function in guard cells. Plant Physiol, 2006, 141: 1316-27
[75] Yoshida R, Umezawa T, Mizoguchi T, et al. The regulatory domain of SRK2E/OST1/SnRK2.6 interacts with ABI1 and integrates abscisic acid (ABA) and osmotic stress signals controlling stomatal closure in *Arabidopsis*. J Biol Chem, 2006, 281: 5310-8
[76] Hsu PK, Takahashi Y, Merilo E, et al. Raf-like kinases and receptor-like (pseudo)kinase GHR1 are required for stomatal vapor pressure difference response. Proc Natl Acad Sci USA, 2021, 118: e2107280118
[77] Scharwies JD, Dinneny JR. Water transport, perception, and response in plants. J Plant Res, 2019, 132: 311-24
[78] Cai S, Chen G, Wang Y, et al. Evolutionary conservation of ABA signaling for stomatal closure. Plant Physiol, 2017, 174: 732-47
[79] Franks PJ, Britton-Harper ZJ. No evidence of general CO_2 insensitivity in ferns: one stomatal control mechanism for all land plants? New Phytol, 2016, 211: 819-27
[80] Hõrak H, Kollist H, Merilo E. Fern stomatal responses to ABA and CO_2 depend on species and growth conditions. Plant Physiol, 2017, 174: 672-9
[81] Clark JW, Harris BJ, Hetherington AJ, et al. The origin and evolution of stomata. Curr Biol, 2022, 32: R539-53
[82] Franks PJ, Farquhar GD. The mechanical diversity of stomata and its significance in gas-exchange control. Plant Physiol, 2007, 143: 78-87
[83] Fitzsimons PJ, Weyers JDB. Separation and purification of protoplast types from *Commelina communis* L. leaf epidermis. J Exp Bot, 1983, 34: 55-66
[84] Weyers JDB, Fitzsimons PJ, Mansey GM, et al. Guard cell protoplasts – aspects of work with an important new research tool. Physiol Plant, 1983, 58: 331-9
[85] Edwards A, Bowling DJF. Evidence for a CO_2 inhibited proton extrusion pump in the stomatal cells of *Tradescantia virginiana*. J Exp Bot, 1985, 36: 91-8

[86] Brearley J, Venis MA, Blatt MR. The effect of elevated CO_2 concentrations on K^+ and anion channels of *Vicia faba* L. guard cells. Planta, 1997, 203: 145-54
[87] Katsuhara M, Hanba YT. Barley plasma membrane intrinsic proteins (PIP aquaporins) as water and CO_2 transporters. Pflüg Arch Eur J Physiol, 2008, 456: 687-91
[88] Wang C, Hu H, Qin X, et al. Reconstitution of CO_2 regulation of SLAC1 anion channel and function of CO_2-permeable PIP2;1 aquaporin as CARBONIC ANHYDRASE4 interactor. Plant Cell, 2016, 28: 568-82
[89] Hu H, Boisson-Dernier A, Israelsson-Nordström M, et al. Carbonic anhydrases are upstream regulators of CO_2-controlled stomatal movements in guard cells. Nat Cell Biol, 2010, 12: 87-93
[90] Hu H, Rappel WJ, Occhipinti R, et al. Distinct cellular locations of carbonic anhydrases mediate carbon dioxide control of stomatal movements. Plant Physiol, 2015, 169: 1168-78
[91] Xue S, Hu H, Ries A, et al. Central functions of bicarbonate in S-type anion channel activation and OST1 protein kinase in CO_2 signal transduction in guard cell. EMBO J, 2011, 30: 1645-58
[92] Jakobson L, Vaahtera L, Tõldsepp K, et al. Natural variation in *Arabidopsis* Cvi-0 accession reveals an important role of MPK12 in guard cell CO_2 signaling. PloS Biol, 2016, 14: e2000322
[93] Hashimoto-Sugimoto M, Negi J, Monda K, et al. Dominant and recessive mutations in the Raf-like kinase *HT1* gene completely disrupt stomatal responses to CO_2 in *Arabidopsis*. J Exp Bot, 2016, 67: 3251-61
[94] Marten H, Hyun T, Gomi K, et al. Silencing of NtMPK4 impairs CO_2 -induced stomatal closure, activation of anion channels and cytosolic Ca^{2+} signals in *Nicotiana tabacum* guard cells. Plant J, 2008, 55: 698-708
[95] Tõldsepp K, Zhang J, Takahashi Y, et al. Mitogen-activated protein kinases MPK4 and MPK12 are key components mediating CO_2-induced stomatal movements. Plant J, 2018, 96: 1018-35
[96] Hashimoto M, Negi J, Young J, et al. *Arabidopsis* HT1 kinase controls stomatal movements in response to CO_2. Nat Cell Biol, 2006, 8: 391-7
[97] Takahashi Y, Bosmans KC, Hsu PK, et al. Stomatal CO_2/bicarbonate sensor consists of two interacting protein kinases, Raf-like HT1 and non-kinase-activity requiring MPK12/MPK4. Sci Adv, 2022, 8: eabq6161
[98] Hiyama A, Takemiya A, Munemasa S, et al. Blue light and CO_2 signals converge to regulate light-induced stomatal opening. Nat Commun, 2017, 8: 1284
[99] Hayashi M, Sugimoto H, Takahashi H, et al. Raf-like kinases CBC1 and CBC2 negatively regulate stomatal opening by negatively regulating plasma membrane H^+-ATPase phosphorylation in *Arabidopsis*. Photochem Photobiol Sci, 2020, 19: 88-98
[100] Vahisalu T, Kollist H, Wang YF, et al. SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling. Nature, 2008, 452: 487-91
[101] Negi J, Matsuda O, Nagasawa T, et al. CO_2 regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. Nature, 2008, 452: 483-6
[102] Zhang J, Wang N, Miao Y, et al. Identification of SLAC1 anion channel residues required for CO_2/bicarbonate sensing and regulation of stomatal movements. Proc Natl Acad Sci USA, 2018, 115: 11129-37
[103] Dubeaux G, Hsu PK, Ceciliato PH, et al. Deep dive into CO_2-dependent molecular mechanisms driving stomatal responses in plants. Plant Physiol, 2021, 187: 2032-42
[104] Rogers CA, Powell RD, Sharpe PJ. Relationship of temperature to stomatal aperture and potassium accumulation in guard cells of *Vicia faba*. Plant Physiol, 1979, 63: 388-91
[105] Rogers C, Sharpe PJ, Powell RD. Dark opening of stomates of *Vicia faba* in CO_2-free air: effect of temperature on stomatal aperture and potassium accumulation. Plant Physiol, 1980, 65: 1036-8
[106] Ilan N, Moran N, Schwartz A. The role of potassium channels in the temperature control of stomatal aperture. Plant Physiol, 1995, 108: 1161-70
[107] Feller U. Stomatal opening at elevated temperature: an underestimated regulatory mechanism. Gen Appl Plant Physiol, 2006, 32: 19-31
[108] Urban J, Ingwers MW, McGuire MA, et al. Increase in leaf temperature opens stomata and decouples net photosynthesis from stomatal conductance in *Pinus taeda* and *Populus deltoides x nigra*. J Exp Bot, 2017, 68: 1757-67
[109] Urban J, Ingwers M, McGuire MA, et al. Stomatal conductance increases with rising temperature. Plant Signal Behav, 2017, 12: e1356534
[110] Devireddy AR, Arbogast J, Mittler R. Coordinated and rapid whole-plant systemic stomatal responses. New Phytol, 2020, 225: 21-5
[111] Cheng C, Wu Q, Wang M, et al. Maize MITOGEN-ACTIVATED PROTEIN KINASE 20 mediates high-temperature-regulated stomatal movement. Plant Physiol, 2023, 193: 2788-805
[112] Kostaki KI, Coupel-Ledru A, Bonnell VC, et al. Guard cells integrate light and temperature signals to control stomatal aperture. Plant Physiol, 2020, 182: 1404-19
[113] Mao J, Zhang YC, Sang Y, et al. A role for *Arabidopsis* cryptochromes and COP1 in the regulation of stomatal opening. Proc Natl Acad Sci USA, 2005, 102: 12270-5
[114] Wang W, Liu Z, Bao LJ, et al. The RopGEF2-ROP7/ROP2 pathway activated by phyB suppresses red light-induced stomatal opening. Plant Physiol, 2017, 174: 717-31
[115] Li Y, Hui S, Yuan Y, et al. PhyB-dependent phosphorylation of mitogen-activated protein kinase cascade MKK2-MPK2 positively regulates red light-induced stomatal opening. Plant Cell Environ, 2023, 46: 3323-36
[116] Chen K, Li GJ, Bressan RA, et al. Abscisic acid dynamics, signaling, and functions in plants. J Integr Plant Biol, 2020, 62: 2554
[117] Zhang J, Chen X, Song Y, et al. Integrative regulatory mechanisms of stomatal movements under changing climate. J Integr Plant Biol, 2024, 66: 368-93

[118] Tian W, Hou C, Ren Z, et al. A molecular pathway for CO_2 response in *Arabidopsis* guard cells. Nat Commun, 2015, 6: 6057
[119] Zhang M, Zhu C, Duan Y, et al. The intrinsically disordered region from PP2C phosphatases functions as a conserved CO_2 sensor. Nat Cell Biol, 2022, 24: 1029-37
[120] Huang YC, Wu HC, Wang YD, et al. PECTIN METHYLESTERASE34 contributes to heat tolerance through its role in promoting stomatal movement. Plant Physiol, 2017, 174: 748-63
[121] Harrison EL, Arce Cubas L, Gray JE, et al. The influence of stomatal morphology and distribution on photosynthetic gas exchange. Plant J, 2020, 101: 768-79
[122] Casson S, Gray JE. Influence of environmental factors on stomatal development. New Phytol, 2008, 178: 9-23
[123] Dittberner H, Korte A, Mettler-Altmann T, et al. Natural variation in stomata size contributes to the local adaptation of water-use efficiency in *Arabidopsis thaliana*. Mol Ecol, 2018, 27: 4052-65
[124] Hetherington AM, Woodward FI. The role of stomata in sensing and driving environmental change. Nature, 2003, 424: 901-8
[125] Zoulias N, Harrison EL, Casson SA, et al. Molecular control of stomatal development. Biochem J, 2018, 475: 441-54
[126] Larkin JC, Marks MD, Nadeau J, et al. Epidermal cell fate and patterning in leaves. Plant Cell, 1997, 9: 1109-20
[127] Pillitteri LJ, Torii KU. Mechanisms of stomatal development. Annu Rev Plant Biol, 2012, 63: 591-614
[128] Qi X, Torii KU. Hormonal and environmental signals guiding stomatal development. BMC Biol, 2018, 16: 21
[129] Endo H, Torii KU. Stomatal development and perspectives toward agricultural improvement. Cold Spring Harb Perspect Biol, 2019, 11: a034660
[130] Hara K, Kajita R, Torii KU, et al. The secretory peptide gene *EPF1* enforces the stomatal one-cell-spacing rule. Genes Dev, 2007, 21: 1720-5
[131] Franks PJ, W. Doheny-Adams T, Britton-Harper ZJ, et al. Increasing water-use efficiency directly through genetic manipulation of stomatal density. New Phytol, 2015, 207: 188-95
[132] Hara K, Yokoo T, Kajita R, et al. Epidermal cell density is autoregulated via a secretory peptide, EPIDERMAL PATTERNING FACTOR 2 in *Arabidopsis* leaves. Plant Cell Physiol, 2009, 50: 1019-31
[133] Hunt L, Gray JE. The signaling peptide EPF2 controls asymmetric cell divisions during stomatal development. Curr Biol, 2009, 19: 864-9
[134] Kondo T, Kajita R, Miyazaki A, et al. Stomatal density is controlled by a mesophyll-derived signaling molecule. Plant Cell Physiol, 2010, 51: 1-8
[135] Hunt L, Bailey KJ, Gray JE. The signalling peptide EPFL9 is a positive regulator of stomatal development. New Phytol, 2010, 186: 609-14
[136] Shpak ED, McAbee JM, Pillitteri LJ, et al. Stomatal patterning and differentiation by synergistic interactions of receptor kinases. Science, 2005, 309: 290-3
[137] Berger D, Altmann T. A subtilisin-like serine protease involved in the regulation of stomatal density and distribution in *Arabidopsis thaliana*. Genes Dev, 2000, 14: 1119-31
[138] Schluter U. Photosynthetic performance of an *Arabidopsis* mutant with elevated stomatal density (*sdd1-1*) under different light regimes. J Exp Bot, 2003, 54: 867-74
[139] Lee LR, Bergmann DC. The plant stomatal lineage at a glance. J Cell Sci, 2019, 132: jcs228551
[140] Hronková M, Wiesnerová D, Šimková M, et al. Light-induced STOMAGEN-mediated stomatal development in *Arabidopsis* leaves. J Exp Bot, 2015, 66: 4621-30
[141] Casson SA, Franklin KA, Gray JE, et al. Phytochrome B and PIF4 regulate stomatal development in response to light quantity. Curr Biol, 2009, 19: 229-34
[142] Casson SA, Hetherington AM. Phytochrome B is required for light-mediated systemic control of stomatal development. Curr Biol, 2014, 24: 1216-21
[143] Kang CY, Lian HL, Wang FF, et al. Cryptochromes, phytochromes, and COP1 regulate light-controlled stomatal development in *Arabidopsis*. Plant Cell, 2009, 21: 262441
[144] Klermund C, Ranftl QL, Diener J, et al. LLM-domain B-GATA transcription factors promote stomatal development downstream of light signaling pathways in *Arabidopsis thaliana* hypocotyls. Plant Cell, 2016, 28: 646-60
[145] Boccalandro HE, Rugnone ML, Moreno JE, et al. Phytochrome B enhances photosynthesis at the expense of water-use efficiency in *Arabidopsis*. Plant Physiol, 2009, 150: 1083-92
[146] Quarrie SA, Jones HG. Effects of abscisic acid and water stress on development and morphology of wheat. J Exp Bot, 1977, 28: 192-203
[147] Xu Z, Zhou G. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. J Exp Bot, 2008, 59: 3317-25
[148] Kumari A, Jewaria PK, Bergmann DC, et al. *Arabidopsis* reduces growth under osmotic stress by decreasing SPEECHLESS protein. Plant Cell Physiol, 2014, 55: 2037-46
[149] Fanourakis D, Heuvelink E, Carvalho SMP. A comprehensive analysis of the physiological and anatomical components involved in higher water loss rates after leaf development at high humidity. J Plant Physiol, 2013, 170: 890-8
[150] Nejad AR, Van Meeteren U. Stomatal response characteristics of *Tradescantia virginiana* grown at high relative air humidity. Physiol Plant, 2005, 125: 324-32
[151] Aliniaeifard S, Van Meeteren U. Stomatal characteristics and desiccation response of leaves of cut chrysanthemum (*Chrysanthemum morifolium*) flowers grown at high air humidity. Sci Hortic, 2016, 205: 84-9
[152] Aliniaeifard S, Malcolm Matamoros P, Van Meeteren U. Stomatal malfunctioning under low VPD conditions: induced by alterations in stomatal morphology and leaf anatomy or in the ABA signaling? Physiol Plant, 2014, 152: 688-99

[153] Hamanishi ET, Thomas BR, Campbell MM. Drought induces alterations in the stomatal development program in populus. J Exp Bot, 2012, 63: 4959-71
[154] Lake JA, Woodward FI. Response of stomatal numbers to CO_2 and humidity: control by transpiration rate and abscisic acid. New Phytol, 2008, 179: 397-404
[155] Tanaka Y, Sugano SS, Shimada T, et al. Enhancement of leaf photosynthetic capacity through increased stomatal density in *Arabidopsis*. New Phytol, 2013, 198: 757-64
[156] Lampard GR, Macalister CA, Bergmann DC. *Arabidopsis* stomatal initiation is controlled by MAPK-mediated regulation of the bHLH SPEECHLESS. Science, 2008, 322: 1113-6
[157] Tricker PJ, Gibbings JG, Rodríguez López CM, et al. Low relative humidity triggers RNA-directed *de novo* DNA methylation and suppression of genes controlling stomatal development. J Exp Bot, 2012, 63: 3799-813
[158] Tricker PJ, López CMR, Gibbings G, et al. Transgenerational, dynamic methylation of stomata genes in response to low relative humidity. Int J Mol Sci, 2013, 14: 6674-89
[159] Woodward FI, Kelly CK. The influence of CO_2 concentration on stomatal density. New Phytol, 1995, 131: 311-27
[160] Woodward FI. Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. Nature, 1987, 327: 617-8
[161] Teng N, Wang J, Chen T, et al. Elevated CO_2 induces physiological, biochemical and structural changes in leaves of *Arabidopsis thaliana*. New Phytol, 2006, 172: 92-103
[162] Gray JE, Holroyd GH, van der Lee FM, et al. The HIC signalling pathway links CO_2 perception to stomatal development. Nature, 2000, 408: 713-6
[163] Chen X, Goodwin SM, Boroff VL, et al. Cloning and characterization of the *WAX2* gene of *Arabidopsis* involved in cuticle membrane and wax production. Plant Cell, 2003, 15: 1170-85
[164] Aharoni A, Dixit S, Jetter R, et al. The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in *Arabidopsis*. Plant Cell, 2004, 16: 2463-80
[165] Engineer CB, Ghassemian M, Anderson JC, et al. Carbonic anhydrases, EPF2 and a novel protease mediate CO_2 control of stomatal development. Nature, 2014, 513: 246-50
[166] Haus MJ, Li M, Chitwood DH, et al. Long-distance and trans-generational stomatal patterning by CO_2 across *Arabidopsis* organs. Front Plant Sci, 2018, 9: 1714
[167] Lv C, Hu Z, Wei J, et al. Transgenerational effects of elevated CO_2 on rice photosynthesis and grain yield. Plant Mol Biol, 2022, 110: 413-24
[168] Sugiura D, Yin W, Kono M, et al. Exploring the responses of crop photosynthesis to CO_2 elevation at the molecular, physiological, and morphological levels toward increasing crop production. Crop Environ, 2024, 3: 75-83
[169] Crawford AJ, McLachlan DH, Hetherington AM, et al. High temperature exposure increases plant cooling capacity. Curr Biol, 2012, 22: R396-7
[170] Lau OS, Song Z, Zhou Z, et al. Direct control of SPEECHLESS by PIF4 in the high-temperature response of stomatal development. Curr Biol, 2018, 28: 1273-80.e3
[171] Parkhurst DF. The adaptive significance of stomatal occurrence on one or both surfaces of leaves. J Ecol, 1978, 66: 367
[172] Mott KA, Gibson AC, O'leary JW. The adaptive significance of amphistomatic leaves. Plant Cell Environ, 1982, 5: 455-60
[173] Mott KA, O'leary JW. Stomatal behavior and CO_2 exchange characteristics in amphistomatous leaves. Plant Physiol, 1984, 74: 47-51
[174] Parkhurst DF, Mott KA. Intercellular diffusion limits to CO_2 uptake in leaves: studies in air and helox. Plant Physiol, 1990, 94: 1024-32
[175] Drake PL, Boer HJ, Schymanski SJ, et al. Two sides to every leaf: water and CO_2 transport in hypostomatous and amphistomatous leaves. New Phytol, 2019, 222: 1179-87
[176] Xiong D, Flexas J. From one side to two sides: the effects of stomatal distribution on photosynthesis. New Phytol, 2020, 228: 1754-66
[177] Goh C, Dietrich P, Steinmeyer R, et al. Parallel recordings of photosynthetic electron transport and K^+-channel activity in single guard cells. Plant J, 2002, 32: 623-30
[178] Goh CH, Oku T, Shimazaki K. Properties of proton pumping in response to blue light and fusicoccin in guard cell protoplasts isolated from adaxial epidermis of *Vicia* leaves. Plant Physiol, 1995, 109: 187-94
[179] Dale JE. Investigations into the stornatal physiology of upland cotton. Ann Bot, 1961, 25: 39-52
[180] Travis AJ, Mansfield TA. Light saturation of stomatal opening on the adaxial and abaxial epidermis of *Commelina communis*. J Exp Bot, 1981, 32: 1169-79
[181] Pemadasa MA. Abaxial and adaxial stomatal responses to light of different wavelengths and to phenylacetic acid on isolated epidermes of *Commelina communis* L. J Exp Bot, 1982, 33: 92-9
[182] Mott KA, Peak D. Effects of the mesophyll on stomatal responses in amphistomatous leaves. Plant Cell Environ, 2018, 41: 2835-43
[183] Muir CD. Making pore choices: repeated regime shifts in stomatal ratio. Proc R Soc B Biol Sci, 2015, 282: 20151498
[184] James SA, Bell DT. Influence of light availability on leaf structure and growth of two *Eucalyptus globulus* ssp. *globulus* provenances. Tree Physiol, 2000, 20: 1007-18
[185] Willmer C, Fricker M. The distribution of stomata [M]// Stomata. Dordrecht: Springer Netherlands, 1996: 12-35
[186] Pemadasa MA. Movements of abaxial and adaxial stomata. New Phytol, 1979, 82: 69-80
[187] Lake JA, Woodward FI, Quick WP. Long-distance CO_2 signalling in plants. J Exp Bot, 2002, 53: 183-93
[188] Lawson T, Von Caemmerer S, Baroli I. Photosynthesis and stomatal behaviour[M]// Lüttge UE, Beyschlag W, Büdel B, et al. Progress in Botany 72. Berlin, Heidelberg: Springer Berlin Heidelberg, 2010: 265-304
[189] McAusland L, Vialet-Chabrand S, Davey P, et al. Effects

of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency. New Phytol, 2016, 211: 1209-20
[190] Lawson T, Blatt MR. Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. Plant Physiol, 2014, 164: 1556-70
[191] Perdomo JA, Capó-Bauçà S, Carmo-Silva E, et al. Rubisco and rubisco activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit. Front Plant Sci, 2017, 8: 490
[192] Wang Y, Wang Y, Tang Y, et al. Stomata conductance as a goalkeeper for increased photosynthetic efficiency. Curr Opin Plant Biol, 2022, 70: 102310
[193] 许大全. 光合作用学[M]. 北京: 科学出版社, 2013
[194] 许大全. 光合作用气孔限制分析中的一些问题. 植物生理学通讯, 1997, 33: 241-4
[195] Jones HG. Partitioning stomatal and non-stomatal limitations to photosynthesis. Plant Cell Environ, 1985, 8: 95-104
[196] Wong SC, Cowan IR, Farquhar GD. Stomatal conductance correlates with photosynthetic capacity. Nature, 1979, 282: 424-6
[197] Mott KA. Do stomata respond to CO_2 concentrations other than intercellular? Plant Physiol, 1988, 86: 200-3
[198] Roelfsema MRG, Hanstein S, Felle HH, et al. CO_2 provides an intermediate link in the red light response of guard cells: CO_2 and the red light response of guard cells. Plant J, 2002, 32: 65-75
[199] Von Caemmerer S, Lawson T, Oxborough K, et al. Stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco with reduced amounts of rubisco. J Exp Bot, 2004, 55: 1157-66
[200] Baroli I, Price GD, Badger MR, et al. The contribution of photosynthesis to the red light response of stomatal conductance. Plant Physiol, 2008, 146: 323-4
[201] Raschke K. Stomatal action. Annu Rev Plant Physiol, 1975, 26: 309-40
[202] Farquhar GD, Sharkey TD. Stomatal conductance and photosynthesis. Annu Rev Plant Physiol, 1982, 33: 317-45
[203] Sharkey TD, Raschke K. Separation and measurement of direct and indirect effects of light on stomata. Plant Physiol, 1981, 68: 33-40
[204] Heath OVS, Russell J. Studies in stomatal behaviour: vi. an investigation of the light responses of wheat stomata with the attempted elimination of control by the mesophyll. J Exp Bot, 1954, 5: 1-15
[205] Lee J, Bowling DJF. Effect of the mesophyll on stomatal opening in *Commelina communis*. J Exp Bot, 1992, 43: 951-7
[206] Lee J, Bowling D. Influence of the mesophyll on stomatal opening. Funct Plant Biol, 1995, 22: 357
[207] Lee J, Bowling DJF. The effect of a mesophyll factor on the swelling of guard cell protoplasts of *Commelina communis* L. J Plant Physiol, 1993, 142: 203-7
[208] Olsen RL, Pratt RB, Gump P, et al. Red light activates a chloroplast-dependent ion uptake mechanism for stomatal opening under reduced CO_2 concentrations in *Vicia* spp. New Phytol, 2002, 153: 497-508
[209] Mott KA, Sibbernsen ED, Shope JC. The role of the mesophyll in stomatal responses to light and CO_2. Plant Cell Environ, 2008, 31: 1299-306
[210] Fujita T, Noguchi K, Terashima I. Apoplastic mesophyll signals induce rapid stomatal responses to CO_2 in *Commelina communis*. New Phytol, 2013, 199: 395-406
[211] Fujita T, Noguchi K, Ozaki H, et al. Confirmation of mesophyll signals controlling stomatal responses by a newly devised transplanting method. Funct Plant Biol, 2019, 46: 467
[212] Mott KA. Opinion: Stomatal responses to light and CO_2 depend on the mesophyll. Plant Cell Environ, 2009, 32: 1479-86
[213] Mott KA, Berg DG, Hunt SM, et al. Is the signal from the mesophyll to the guard cells a vapour-phase ion? Plant Cell Environ, 2014, 37: 1184-91
[214] Sibbernsen E, Mott KA. Stomatal responses to flooding of the intercellular air spaces suggest a vapor-phase signal between the mesophyll and the guard cells. Plant Physiol, 2010, 153: 1435-42
[215] Santos MG, Davey PA, Hofmann TA, et al. Stomatal responses to light, CO_2, and mesophyll tissue in *Vicia faba* and *Kalanchoë fedtschenkoi*. Front Plant Sci, 2021, 12: 740534
[216] McAdam SAM, Brodribb TJ. Stomatal innovation and the rise of seed plants. Ecol Lett, 2012, 15: 1-8
[217] Lu P, Outlaw WH Jr, Smith BG, et al. A new mechanism for the regulation of stomatal aperture size in intact leaves (accumulation of mesophyll-derived sucrose in the guard-cell wall of *Vicia faba*). Plant Physiol, 1997, 114: 109-18
[218] Outlaw WH, De Vlieghere-He X. Transpiration rate. An important factor controlling the sucrose content of the guard cell apoplast of broad bean. Plant Physiol, 2001, 126: 1716-24
[219] Hedrich R, Marten I. Malate-induced feedback regulation of plasma membrane anion channels could provide a CO_2 sensor to guard cells. EMBO J, 1993, 12: 897-901
[220] Hedrich R, Marten I, Lohse G, et al. Malate-sensitive anion channels enable guard cells to sense changes in the ambient CO_2 concentration. Plant J, 1994, 6: 741-8
[221] Lee M, Choi Y, Burla B, et al. The ABC transporter AtABCB14 is a malate importer and modulates stomatal response to CO_2. Nat Cell Biol, 2008, 10: 1217-23
[222] Głowacka K, Kromdijk J, Kucera K, et al. Photosystem II subunit S overexpression increases the efficiency of water use in a field-grown crop. Nat Commun, 2018, 9: 868
[223] Still CJ, Berry JA, Collatz GJ, et al. Global distribution of C_3 and C_4 vegetation: carbon cycle implications. Glob Biogeochem Cycles, 2003, 17: 1006
[224] Sage RF, Sage TL, Kocacinar F. Photorespiration and the evolution of C_4 photosynthesis. Annu Rev Plant Biol, 2012, 63: 19-47
[225] Way DA, Katul GG, Manzoni S, et al. Increasing water use efficiency along the C_3 to C_4 evolutionary pathway: a stomatal optimization perspective. J Exp Bot, 2014, 65:

3683-93
[226] Ghannoum O. How can we breed for more water use-efficient sugarcane? J Exp Bot, 2016, 67: 557-9
[227] Cano FJ, Sharwood RE, Cousins AB, et al. The role of leaf width and conductances to CO_2 in determining water use efficiency in C_4 grasses. New Phytol, 2019, 223: 1280-95
[228] Dubbe DR, Farquhar GD, Raschke K. Effect of abscisic acid on the gain of the feedback loop involving carbon dioxide and stomata. Plant Physiol, 1978, 62: 413-7
[229] Huxman TE, Monson RK. Stomatal responses of C_3, C_3-C_4 and C_4 flaveria species to light and intercellular CO_2 concentration: implications for the evolution of stomatal behaviour. Plant Cell Environ, 2003, 26: 313-22
[230] Maherali H, Reid CD, Polley HW, et al. Stomatal acclimation over a subambient to elevated CO_2 gradient in a C_3/C_4 grassland. Plant Cell Environ, 2002, 25: 557-66
[231] Akita S, Moss DN. Differential stomatal response between C_3 and C_4 species to atmospheric CO_2 concentration and light. Crop Sci, 1972, 12: 789-93
[232] Zhen S, Bugbee B. Steady-state stomatal responses of C_3 and C_4 species to blue light fraction: interactions with CO_2 concentration. Plant Cell Environ, 2020, 43: 3020-32
[233] Bernardo EL, Sales CRG, Cubas LA, et al. A comparison of stomatal conductance responses to blue and red light between C_3 and C_4 photosynthetic species in three phylogenetically-controlled experiments. Front Plant Sci, 2023, 14: 1253976
[234] Taylor SH, Franks PJ, Hulme SP, et al. Photosynthetic pathway and ecological adaptation explain stomatal trait diversity amongst grasses. New Phytol, 2012, 193: 387-96
[235] Zhao YY, Lyu MA, Miao F, et al. The evolution of stomatal traits along the trajectory toward C_4 photosynthesis. Plant Physiol, 2022, 190: 441-58
[236] Cockburn W, Ting IP, Sternberg LO. Relationships between stomatal behavior and internal carbon dioxide concentration in crassulacean acid metabolism plants. Plant Physiol, 1979, 63: 1029-32
[237] Spalding MH, Stumpf DK, Ku MSB, et al. Crassulacean acid metabolism and diurnal variations of internal CO_2 and O_2 concentrations in *Sedum praealtum* DC. Funct Plant Biol, 1979, 6: 557-67
[238] Males J, Griffiths H. Stomatal biology of CAM plants. Plant Physiol, 2017, 174: 550-60
[239] Von Caemmerer S, Griffiths H. Stomatal responses to CO_2 during a diel crassulacean acid metabolism cycle in *Kalanchoe daigremontiana* and *Kalanchoe pinnata*. Plant Cell Environ, 2009, 32: 567-76
[240] Mawson BT, Zaugg MW. Modulation of light-dependent stomatal opening in isolated epidermis following induction of crassulacean acid metabolism in *Mesembryanthemum crystallinum* L. J Plant Physiol, 1994, 144: 740-6
[241] Lee DM, Assmann SM. Stomatal responses to light in the facultative crassulacean acid metabolism species, *Portulacaria afra*. Physiol Plant, 1992, 85: 35-42
[242] Abraham PE, Yin H, Borland AM, et al. Transcript, protein and metabolite temporal dynamics in the CAM plant *Agave*. Nat Plants, 2016, 2: 16178
[243] Lange OL, Medina E. Stomata of the CAM plant *Tillandsia recurvata* respond directly to humidity. Oecologia, 1979, 40: 357-63
[244] Faralli M, Matthews J, Lawson T. Exploiting natural variation and genetic manipulation of stomatal conductance for crop improvement. Curr Opin Plant Biol, 2019, 49: 1-7
[245] Doheny-Adams T, Hunt L, Franks PJ, et al. Genetic manipulation of stomatal density influences stomatal size, plant growth and tolerance to restricted water supply across a growth carbon dioxide gradient. Philos Trans R Soc B Biol Sci, 2012, 367: 547-55
[246] Hughes J, Hepworth C, Dutton C, et al. Reducing stomatal density in barley improves drought tolerance without impacting on yield. Plant Physiol, 2017, 174: 776-87
[247] Caine RS, Yin X, Sloan J, et al. Rice with reduced stomatal density conserves water and has improved drought tolerance under future climate conditions. New Phytol, 2019, 221: 371-84
[248] Jiang Q, Yang J, Wang Q, et al. Overexpression of MdEPF2 improves water use efficiency and reduces oxidative stress in tomato. Environ Exp Bot, 2019, 162: 321-32
[249] Wang C, Liu S, Dong Y, et al. PdEPF1 regulates water-use efficiency and drought tolerance by modulating stomatal density in poplar. Plant Biotechnol J, 2016, 14: 849-60
[250] Liu Y, Qin L, Han L, et al. Overexpression of maize SDD1(ZmSDD1) improves drought resistance in *Zea mays* L. by reducing stomatal density. Plant Cell Tissue Organ Cult Pctoc, 2015, 122: 147-59
[251] Morales-Navarro S, Pérez-Díaz R, Ortega A, et al. Overexpression of a *SDD1-like* gene from wild tomato decreases stomatal density and enhances dehydration avoidance in *Arabidopsis* and cultivated tomato. Front Plant Sci, 2018, 9: 940
[252] Gray J, Dunn J. Optimizing crop plant stomatal density to mitigate and adapt to climate change. Cold Spring Harb Perspect Biol, 2023, 16: a041672
[253] Wang Y, Noguchi K, Ono N, et al. Overexpression of plasma membrane H^+-ATPase in guard cells promotes light-induced stomatal opening and enhances plant growth. Proc Natl Acad Sci USA, 2014, 111: 533-8
[254] Zhang M, Wang Y, Chen X, et al. Plasma membrane H^+-ATPase overexpression increases rice yield via simultaneous enhancement of nutrient uptake and photosynthesis. Nat Commun, 2021, 12: 735
[255] Papanatsiou M, Petersen J, Henderson L, et al. Optogenetic manipulation of stomatal kinetics improves carbon assimilation, water use, and growth. Science, 2019, 363: 1456-9
[256] Zheng S, Ye C, Lu J, et al. Improving the rice photosynthetic efficiency and yield by editing OsHXK1 via CRISPR/Cas9 system. Int J Mol Sci, 2021, 22: 9554
[257] Daloso DM, Williams TCR, Antunes WC, et al. Guard cell-specific upregulation of sucrose synthase 3 reveals that the role of sucrose in stomatal function is primarily energetic. New Phytol, 2016, 209: 1470-83