GORK

Super-synchronised stomatal control approachable by gene editing

Enhanced photosynthesis and growth while preserving water status (2xWUE)

Evaluation and Licensing Opportunities

For further information on this technology and evaluation / licensing opportunities please contact:

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Patent Literature

20.665
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23.700
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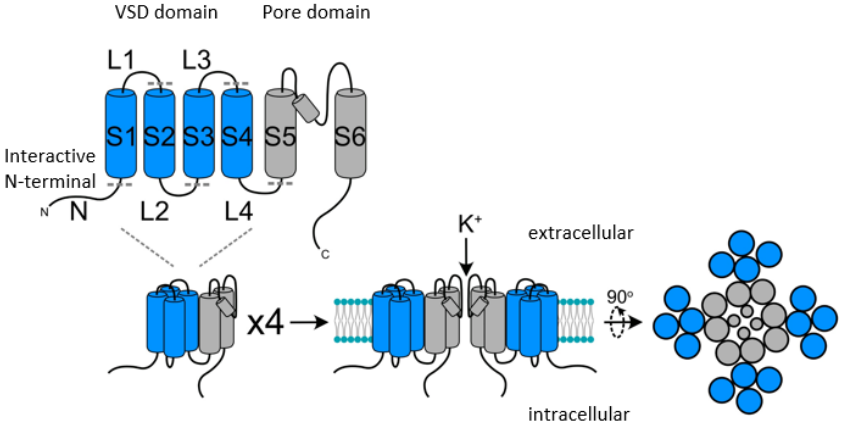
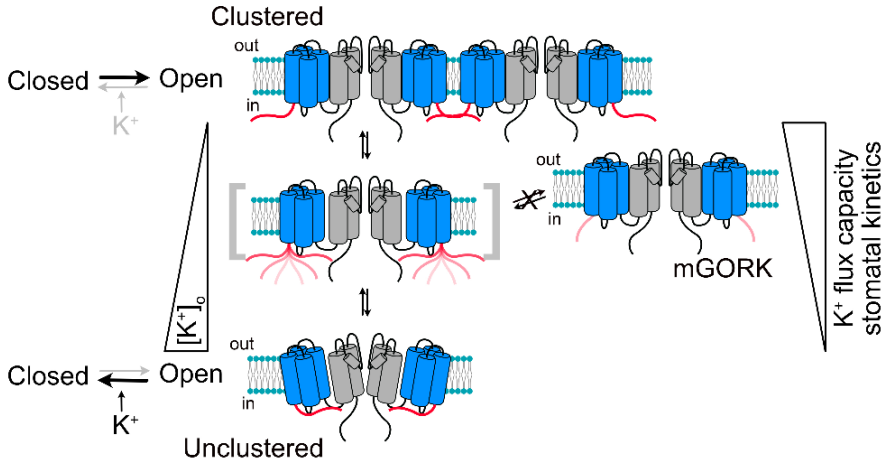
Stomata allow CO_2 uptake for photosynthetic carbon assimilation but often at the expense of water loss via transpiration. Stomatal opening occurs in response to changes in atmospheric CO_2 concentration, ambient light, atmospheric relative humidity and ABA. Approaches to enhance water-use efficiency in plants generally come up against this inherent trade-off between carbon assimilation and water use. Prof Mike Blatt and colleagues at the University of Glasgow have devised a gene-editable approach that accelerates the kinetics of stomatal opening and closure, thus promoting a rapid transition to carbon assimilation under high-light light intensities, while maintaining plant water status when carbon demand is low.

In natural crop growing conditions with fluctuating light, e.g. varying cloud cover or within crop canopy conditions, stomatal opening often lags behind the changing micro-environment with consequent growth penalties to the crop. In the **BLINK** technology (**PBL Tech Id 19.651**) invented by Profs Mike Blatt and John Christie the transgenic expression of a synthetic light-gated potassium channel in guard cells surrounding the stomatal pores enhances the solute fluxes that drive effects on stomatal aperture. The novel K^+ channel, BLINK1, was constructed by fusing the plant LOV2-Jα photosensory module to the miniature K^+ channel, Kcv, of the *Chlorella* virus PBCV. This results in a synthetic, blue light-gated channel. When expressed in plants, and particularly in the stomatal guard cells, this results both in accelerated stomatal opening upon light irradiation and also in faster closing following a return to darker conditions. With the more responsive, accelerated stomatal opening and closure afforded by expressing BLINK channels, stomatal aperture can be brought in synchrony with the environment, thus enhancing carbon-assimilation and water use, proving the concept that **stomatal synchrony can be beneficially modified**.

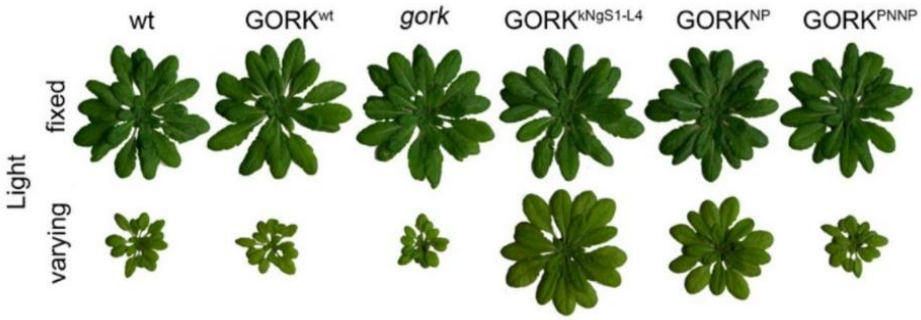
Now Prof Blatt and his team have shown that clustering of the **GORK** ion channel, which mediates K^+ efflux for stomatal closure, is mediated by the voltage sensor domains (gVSDs) that surround the central pore of each GORK K^+ channel. They create a 'sensory antenna' that affects channel gating. Clustering arises from a unique set of interactions between charged surfaces of neighbouring voltage sensor domains. Manipulating these domains to change their charge pattern – which can readily be done by mutation or gene editing – not only uncouples GORK activity from clustering but also promotes K^+ flux. This is because the binding behaviour of the VSDs causes clustering in one configuration and closing of the GORK K^+ channel in another, as will be explained. Mutant Arabidopsis plants with non-interacting GORK VSDs have highly significantly faster decline in stomatal conductance on transit to dark and higher steady-state stomatal conductance in the light – i.e. a much more responsive stomatal response. The research has shown that such modifications **optimize stomatal aperture**, **enhance water use efficiency without biomass penalty** and indeed ***improve*** **photosynthetic carbon assimilation and growth**.

GORK ion channels are formed by tetramers of GORK proteins. Each GORK comprises 6 transmembrane domains, four making up the voltage sensor domain and two C-terminal domains making up one quarter of the ion channel pore. Using sequential domain substitution and mutation studies in the gVSDs, the inventors show that GORK clusters occur by the interaction between a cytosolic region of around 40 aa in the GORK N-terminal and a neighbouring GORK protein. This interaction (binding) is based on a charge-based zipper-like interaction of this N-terminal gVSD sequence on with a complementary cytosolic surface elsewhere in the GORK protein.





Model of GORK channel clustering and K^+ flux activity (gating): Potassium encourages the cytosolic VSD N-terminals (red) to bind to the main channel core, closing the pore. In the absence of K^+, gVSDs are available for clustering. Mutant/modified GORK (mGORK) channels with non-binding VSDs do not enter the 'locked-closed' state and have enhanced K^+ flux and better stomatal kinetics.



Plants with non-binding gVSDs have enhanced growth under non-constant light conditions: Growth over five weeks of: wt, wt expressing GORK, gork null mutants, two different lines with non-binding gVSDs and a line $GORK^{PNNP}$ in which positive and negative residues of gVSD had been swapped. The plants were grown either in a fixed light regime or a fluctuating daylight period varying between 10 and 220 µmol $m^{-2}s^{-1}$. In both regimes soil moisture was limited at 8% through a 5-week trial growth period. In the "field realistic", varying light conditions the GORK binding-compromised plants produced **3x dry biomass** than other plants and this translates to a **doubling of water use efficiency**.

GORK proteins, like other Kv channels such as SKOR, are highly conserved in the plant kingdom. Comparison of the cytosolic N-termini of GORKs from a diverse range of plant crop species, including **major crops**, indicates a well-conserved pattern of alternating charges (the "zipper") beyond the first 20 amino acids. The University of Glasgow research shows that simple changes that affect the pattern of charged amino acids in the GORK VSD can be made, by methods such as mutation or gene editing, to favourably affect the function of the GORK channel.

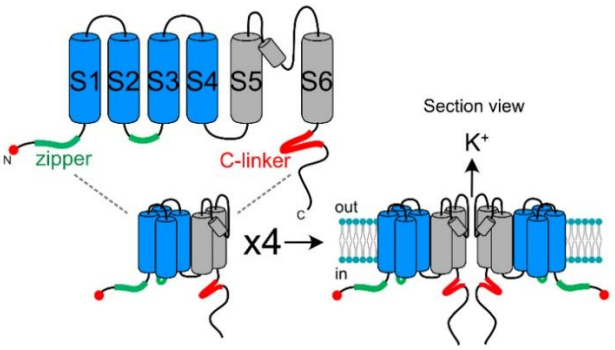
Alignment of GORK N-terminal domains (residues 24-59) from various plant species, including crops such as maize, wheat, rice, oilseed rape etc, showing the pattern of alternating positive (blue) and negatively (red) charged residues

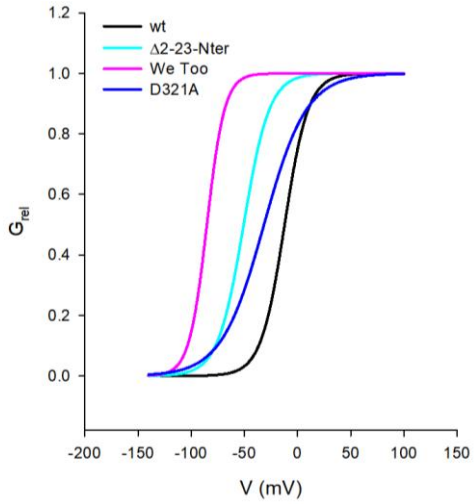
UPDATE July 2023 - PBL Tech Id 23.700

New, alternative mutation strategies

Further studies by Mike Blatt and his team have demonstrated other mutation strategies that may work as well or even better than altering the amino acid charge pattern in the VSD "zipper" region of GORK. Single residue changes in the centrally located C-linker region also affect the voltage gating properties of the GORK channel and thus represent a simpler approach. In addition, deletion of sections of the N-terminal are also effective, again a **simpler approach than altering the zipper charge pattern**. And combination of mutation/deletion of the N-terminal with the C-linker mutations gives the strongest effect seen so far on channel-gating and the most rapid and sensitive stomatal response. This leads to an understanding of the working model of the channel whereby the N-terminal and the C-linker interact to close, and hold closed, the ion-channel.

GORK and its C-linker region





Effect of the combination of N-terminal and C-linker mutants on channel gating

PBL has filed a follow-up patent application (PBL Tech Id 23.700) describing these alternative mutation strategies, which of course are also amenable to modification by genome editing approaches.

This GORK improvement represents the **700th technology** coded by PBL.

The GORK stomatal control technology is patented by PBL on behalf of the University of Glasgow. For more information or licensing interest, please contact PBL.

References:

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