Title:

The transient expression of recombinant proteins in plant cell packs facilitates stable isotope labeling for NMR spectroscopy

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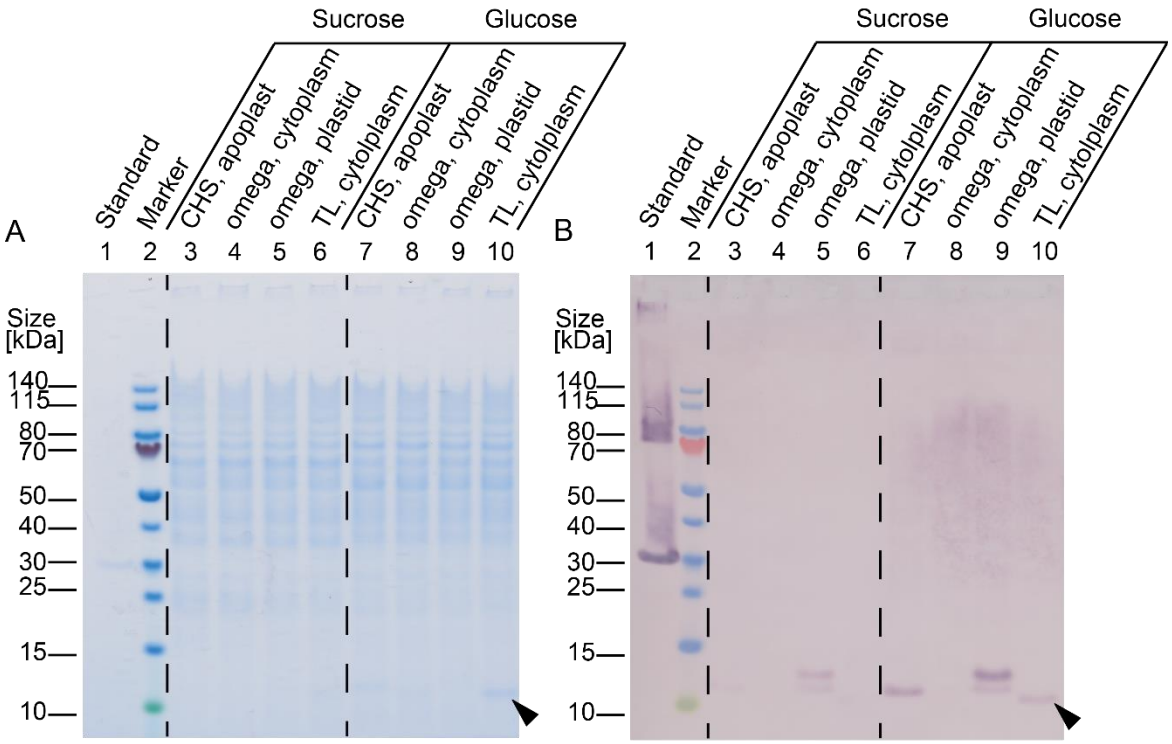
Key words: alternative expression host; defined cultivation media; isotope labeling; plant cell culture; structural analysis

Supplementary materials



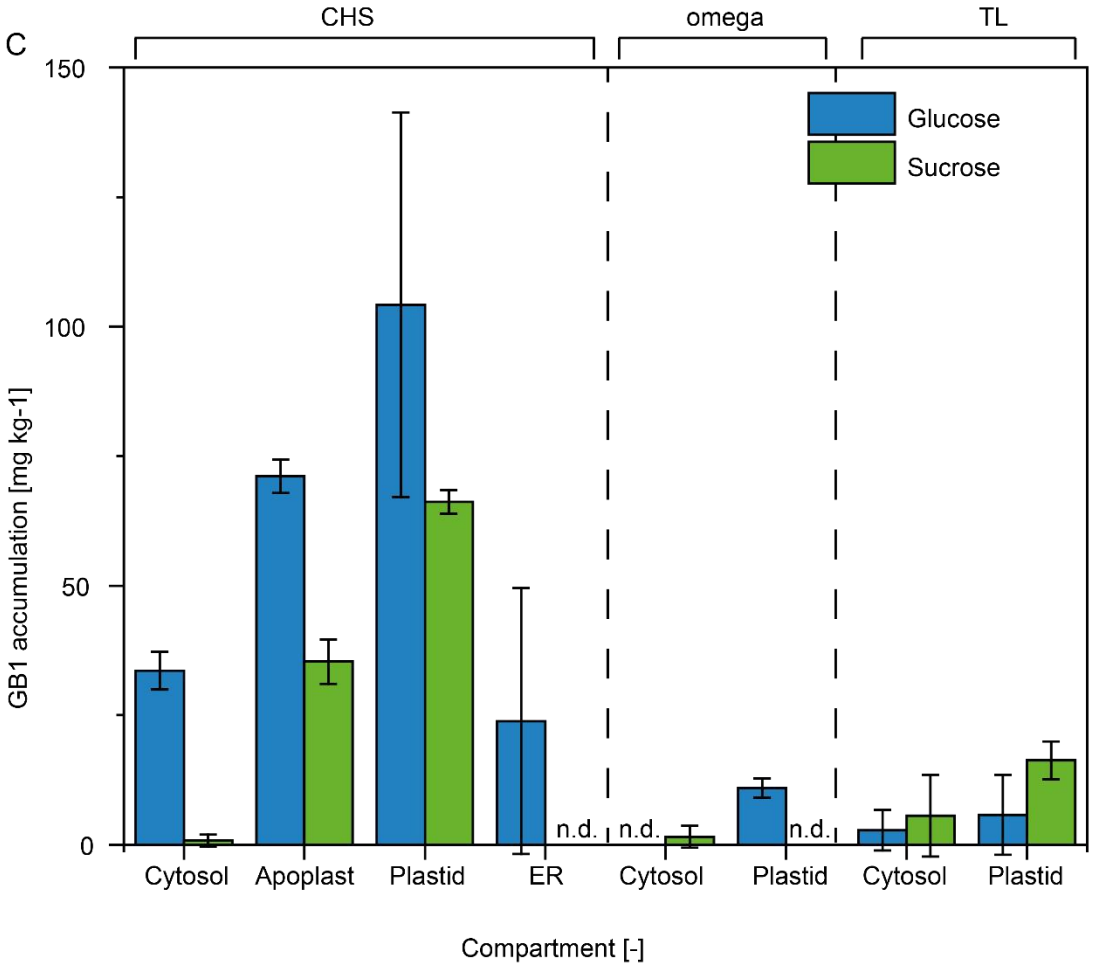


Figure S1: GB1 expression in different plant cell compartments in dependence of the carbon source used during BY-2 cell cultivation. A. Exemplary extracts of PCPs expressing GB1 targeted to different sub-cellular compartments were analyzed by LDS gel electrophoresis and subsequent Coomassie staining. B. The same samples as in A were also analyzed by western blotting using a rabbit anti-His_6 primary antibody and an alkaline phosphatase-labeled goat anti-rabbit secondary antibody to detect the C-terminal His_6-tag that was part of each GB1 variant. C. GB1 concentrations in PCP extracts were quantified via dot blot against a series of His_6-tag GB1 standard concentrations. Error bars indicate the standard deviation ($n \geq 2$). BY-2 cells used for GB1 expression were cultivated using either sucrose or glucose as the sole carbon source. BY-2 cells were incubated for 72 h following infiltration with *A. tumefaciens*. The carbon source concentration was 30 g L^{-1} which corresponded to 166.5 mM and 87.54 mM for glucose and sucrose respectively. CHS – chalcone synthase UTR; ER – endoplasmic reticulum; n.d. – not detected; omega – omega prime sequence of Tobacco mosaic virus; TL – leader sequence of Tobacco etch virus.

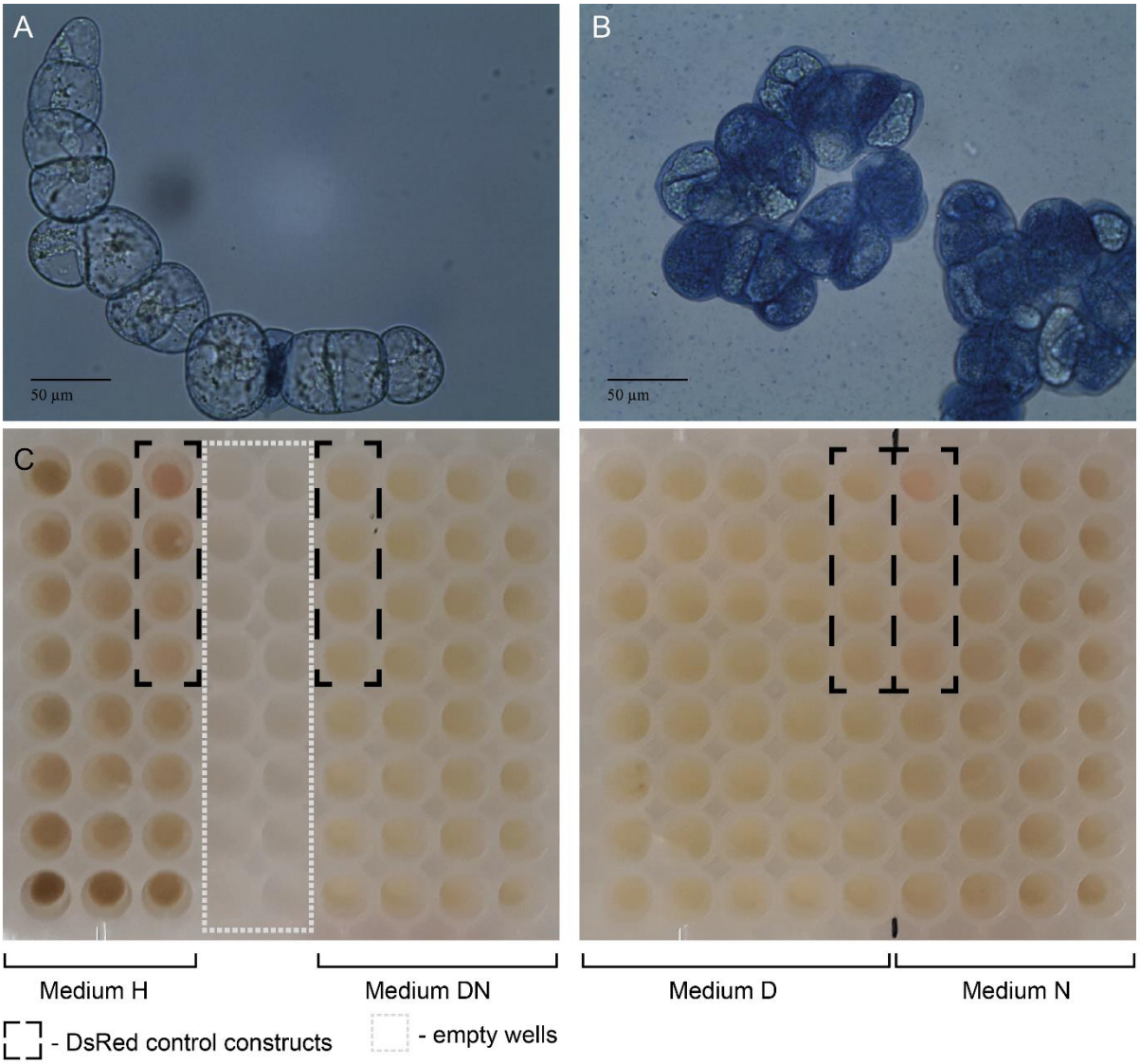


Figure S2: Cell viability in media and PCPs. A. Microscopic image of BY-2 cells grown in medium H (control) after Evans blue staining at 40-fold magnification. The image shows cells associate like "pearls-on-a-string" as a moderate form of aggregation commonly observed for this cell line. B. Same cells as in A. but after 2 h of incubation in ~1.5 M sodium chloride. Intensive blue staining indicates cells with compromised cell membrane integrity. C. Photograph of PCPs prepared from BY-2 cells cultivated in media containing isotope-labeled substrates (D, N, DN) or control medium (H) (**Table 2**) and cast in 96-well filter plates. The PCPs are shown 4 days post-infiltration (dpi) with *A. tumefaciens*. PCP browning indicating a loss of viability was observed at 4 dpi only in the isotope-free medium (H). All

non-control PCPs were infiltrated with *A. tumefaciens* carrying pTRAc vector with T-DNA coding for plastid-targeted GB1. D – MS medium prepared with 50% D_2O; DN – MS medium prepared with 50% D_2O and ^{15}N ammonium nitrate; H – Regular MS medium without labeled components; N – MS medium prepared with ^{15}N ammonium nitrate.

Table S1: Comparison of GB1 accumulation levels in PCPs prepared from BY-2 cells grown in different cultivation media.

Cultivation setting 1	$n_{\text{cultivation setting 1}}$	Cultivation setting 2	$n_{\text{cultivation setting 2}}$	alpha	p-value
H	14	D	12	0.05	<0.0001
H	14	N	14	0.05	0.0085
H	14	DN	14	0.05	<0.0001
D	12	N	14	0.05	<0.0001
D	12	DN	14	0.05	<0.0001
N	14	DN	14	0.05	0.0055

Two-sided two-sample t-tests were used to compare GB1 accumulation levels between PCPs. We used the Shapiro-Wilk test for normality and the F-test for equal variances. A Welch correction was applied for the comparison of samples with unequal variance. D – MS medium prepared with 50% D_2O; DN – MS medium prepared with 50% D_2O and ^{15}N ammonium nitrate; H – Regular MS medium without labeled components; N – MS medium prepared with ^{15}N ammonium nitrate. The medium composition is listed in **Table 2**.