2′,3′-cAMP treatment mimics abiotic stress response

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Abstract

For many years, 2′,3′-cyclic adenosine monophosphate (2′,3′-cAMP), a positional isomer of the second messenger 3′,5′-cAMP, has not received enough attention. Recent studies have reported that 2′,3′-cAMP exists in plants and might be involved in stress signaling because 1) its level increases upon wounding and 2) it was shown to participate in stress granule formation. Although 2′,3′-cAMP is known as RNA-degradation product, the effect of its accumulation in the cell remains unknown. Here, we unprecedentedly evaluate responses at the transcriptome, metabolome, and proteome levels to the accumulation of 2′,3′-cAMP in Arabidopsis plants. Data revealed that 2′,3′-cAMP is metabolized into adenosine, suggesting that a well-known cyclic nucleotide - adenosine pathway from human cells might exist also in plants. However, the control transcriptomic data indicated that, despite fewer overlaps, responses to 2′,3′-cAMP and to adenosine differ. Analysis of the transcriptome and proteome in response to 2′,3′-cAMP showed similar changes, known as signatures for abiotic stress response. This is further supported by the fact that adding to the role in facilitating of stress granules (SGs) formation, 2′,3′-cAMP induces changes at the level of proteins known as key components of SGs and can also induce movement of processing bodies (PBs) in the cell.

Keywords: 2′,3′-cAMP, abiotic stress, processing bodies, stress response, stress granules

Introduction

To cope with the fluctuating environment, living organisms developed signaling mechanisms to respond quickly and adapt to the changing conditions. Signaling cascades comprise diverse protein and small molecule players, which by series of timely and spatially spaced interactions regulate the activity, localization, and aggregation of downstream targets driving physiological alterations. Cyclic nucleotides comprise a group of important and evolutionary conserved signaling small molecules. In human cells, 3′,5′-cyclic adenosine monophosphate (3′,5′-cAMP) acts as a second messenger downstream of adrenaline and glucagon but upstream of sugar and lipid metabolism. In contrast to 3′,5′-cAMP, its positional isomer—2′,3′-cyclic adenosine monophosphate (2′,3′-cAMP)—has received considerably less attention. In fact, it was only in 2009 that 2′,3′-cAMP was discovered in a biological material (Ren et al., 2009), which led to further functional studies. 2′,3′-cAMP is a product of 3′→5′ degradation (Thompson et al., 1994) and, thus, not surprisingly accumulates under conditions characterized by excessive mRNA decay such as tissue injury (Jackson et al., 2009; Verrier et al., 2012; Van Damme et al., 2014). Although this is also true for other 2′,3′-cNMPs, 2′,3′-cAMP is the most abundant, mostly due to the presence of the mRNA poly(A) tail. In animal cells, high levels of cellular 2′,3′-cAMP are considered toxic and have been linked to mitochondrial dysfunction (Azarashvili et al., 2009). Relatedly, animal cells have a way to efficiently metabolize 2′,3′-cAMP, first to 2′-AMP *via* the activity of the 2′,3′-cyclic nucleotide-3′-phosphodiesterase (CNPase) and subsequently to adenosine (Jackson et al., 2009; Jackson, 2016) which was shown to have protective properties (Jackson, 2016). Conversion of 2′,3′-cAMP to adenosine is discussed as a switch from a toxic to a nontoxic cellular environment.

Like animal cells, in plants, cellular levels of 2′,3′-cAMP were reported to increase under stress treatments such as wounding (Van Damme et al., 2014) or heat and dark conditions (Kosmacz et al., 2018). Recently, 2′,3′-cAMP has been shown to interact with Rbp47b (Kosmacz et al., 2018; Kosmacz and Skirycz, 2020), protein important for stress granule (SG) formation under stress conditions (Kosmacz et al., 2019), supporting its potential function in stress signaling. Since the amount of 2′,3′-cAMP in the cell might be crucial for cellular signaling, to gain more detailed knowledge about the function of 2′,3′-cAMP in plants, we decided to evaluate the response to 2′,3′-cAMP at the metabolic, proteomic, and transcriptomic levels. To obtain a complete picture of

regarding 2′,3′-cAMP treatment, we decided to include adenosine treatment as a control for transcriptome analysis, considering the existence of the 2′,3′-cAMP-adenosine pathway in animal cells. Both compounds were applied as permeable Br-versions to ensure their uptake by the seedlings of *Arabidopsis thaliana*. Overall, the obtained results revealed that (i) 2′,3′-cAMP was up taken by plants and metabolized to adenosine, (ii) 2′,3′-cAMP triggered a specific response at the metabolite, transcriptome, and proteome levels, and (iii) treatment with 2′,3′-cAMP affected the abundance of key SG proteins and induced processing body (PB) movement.

Results

Treatment with 2′,3′-cAMP leads to the accumulation of stress-responsive metabolites

To characterize plant response to the accumulation of 2′,3′-cAMP, we performed feeding experiments by treating Arabidopsis seedlings growing in liquid cultures with 1 µM of membrane permeable analogue of 2′,3′-cAMP, Br-2′,3′-cAMP. Samples were harvested after 15 min, 30 min, 1 h, 6 h, and 24 h of treatment with either mock solution (control samples) or Br-2′,3′-cAMP (treated samples) (Figure 1A). We confirmed the uptake of Br-2′,3′-cAMP using liquid chromatography–mass spectrometry (LC/MS) based metabolomics (Figure 1B). 15 min was sufficient to detect Br-2′,3′-cAMP accumulation in the treated seedlings. The level peaked at 30 min but dropped sharply at 1 h and decreased further at 6 h. Strikingly, no Br-2′,3′-cAMP was detected in the samples taken at 24 h, suggesting a rapid turn-over of the compound. In support of plants up-taking Br-2′,3′-cAMP, the detection of Br-adenosine was possible in treated seedlings where an increase in Br-adenosine was accompanied by a decrease in Br-2′,3′-cAMP, suggesting an active conversion of 2′,3′-cAMP into adenosine. Again, only traces of Br-adenosine were detected in the samples taken at 24 h, suggesting further decay or exclusion. Our data demonstrate that plants can efficiently metabolize exogenously supplied Br-2′,3′-cAMP to Br-adenosine, which suggests the presence of phosphodiesterase activity. Further experiments are required to determine whether this is also true for the endogenously produced 2′,3′-cAMP.

Looking at the whole metabolome (Supplementary Table S1), we detected 151 primary and specialized metabolites, among which 82 were significantly affected by the treatment with Br-2′,3′-cAMP (p-value adj. ≤ 0.05) (Figure 1C, D). Among these, a high number corresponds to the group of dipeptides (29), amino acids (13), or nucleotides (5) (Figure 1C). The majority of significantly affected metabolites were upregulated (Figure 1D). Interestingly, significant

upregulation of RNA-degradation products, such as endogenous 2′,3′-cAMP itself, adenosine 2′-monophosphate, and uric acid, was also observed. This might suggest that the treatment with Br-2′,3′-cAMP mimics the response to stress conditions.

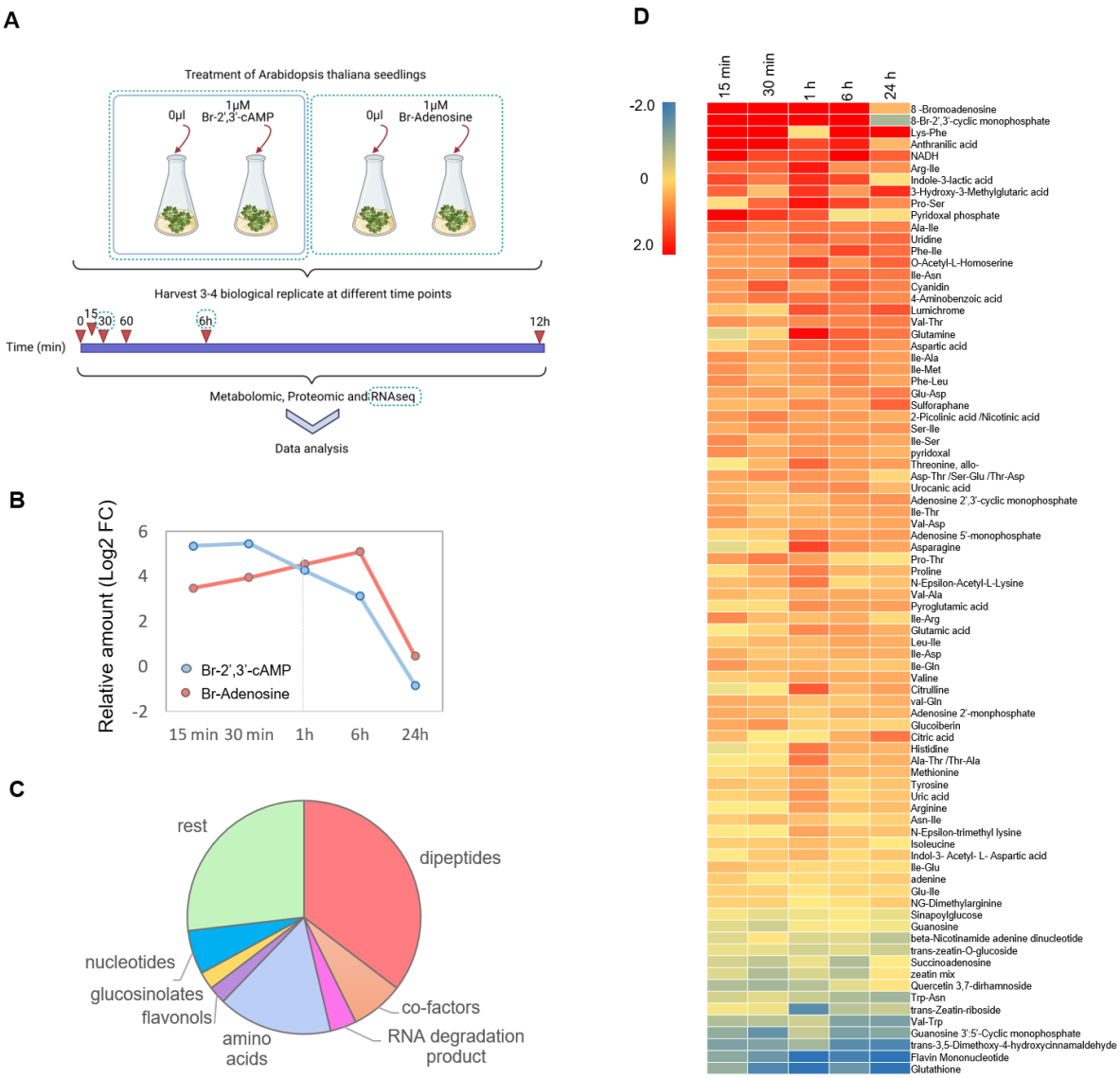

Figure 1. 2′,3′-cAMP induces stress-responsive changes at the metabolome level. **A.** Experimental design. Arabidopsis wild-type plants were treated with mock, 1 µM Br-2′,3′-cAMP or 1 µM Br-adenosine (for RNAseq analysis). 3–4 biological samples were collected at six time points for proteomic and metabolomics analysis: 0, 15 min, 30 min, 1 h, 6 h, and 24 h and only two time points of 15 min and 6 h for transcriptome analysis. Samples were extracted and prepared for

proteomics, metabolomics, and RNAseq analysis. Data were analyzed with a focus on significant 2′,3′-cAMP induced changes. **B.** Change in the amount of Br-2′,3′-cAMP and Br-adenosine levels in the plants treated with 1 µM Br-2′,3′-cAMP. **C.** Groups of metabolites significantly changed upon 2′,3′-cAMP treatment (FDR adjusted *p-value* ≤ 0.05). **D.** Heat map representing overall significant changes at the metabolite level after treatment with Br-2′,3′-cAMP. Data are presented as Log_2 FC (FDR-adjusted *p-value* ≤ 0.05).

Transcriptome analysis revealed that 2′,3′-cAMP mimics an abiotic stress response

To properly interpret the data, the first step was to understand whether the observed changes were due to specific 2′,3′-cAMP treatment and not due to an increase in adenosine in the cell. Hence, an evaluation of the molecular response at the transcriptional level was performed for two time points, including 30 min and 6 h for 2′,3′-cAMP and Br-adenosine treatment. In our analysis, differentially expressed genes (DEGs) were defined as genes significantly (p-value adj. ≤ 0.05) upregulated or downregulated compared to non-treated samples. Thus, we could compare two datasets, which were stated as 2′,3′-cAMP (2′,3′-cAMP vs non-treated) and adenosine (adenosine vs non-treated). The highest number of DEGs corresponding to 2322 was identified in the Br-adenosine experiment (Figure 2A and Supplementary Table 2, 3), where ~3% of genes were upregulated and ~5,5% was downregulated at 30 min of treatment. Lower number of DEGs were identified in 2′,3′-cAMP corresponding to 953 genes (431 being upregulated and 522 being downregulated) to be affected. The situation became more even after 6 h (Figure 2A and Supplementary Table 3), where in both treatments, the number of DEGs was relatively high, being in the range of 2200–3000 genes.

The comparison between responsive but also significantly changed genes in both experiments at 30 min (Figure 2B, C) and 6 h (Supplemental Figure 1) time points revealed that the majority of DEGs were specific to the treatment (Figure 2B, C). An enrichment analysis of the processes (Fisher's exact test with an FDR correction *p-value* < 0.05) was performed using the PANTHER overrepresentation test (PANTHER13.1) with the GO ontology database (Mi et al., 2017). Common upregulated genes for 2′,3′-cAMP and adenosine treatment belonged to the group involved in the cytokinin-activated signaling pathway, hormonal response, and response to salt stress (Supplementary Table 4). For downregulated genes, in an overlap between 2′,3′-cAMP and adenosine, we observed an enrichment of genes involved in heat stress response, acclimation, and response to oxidative stress (Supplementary Table 4, Supplementary Figure 1A, B). For the

specific DEGs induced at 30 min of 2′,3′-cAMP treatment, the performed GO term enrichment analysis revealed the highest fold enrichment for oxylipin biosynthetic process (14), genes involved in JA signaling pathways (13,8), and response to wounding (7) (Figure 2D, Supplementary Table 5, Supplementary Figure 1A, B). Also, 30 min of adenosine treatment showed enrichment in many biological processes, among which (Figure 2D, Supplementary Table 7) syncytium formation (14,5), cell wall modification (11,5), and hormonal signaling (gibberellin mediated signaling pathway) (6,9) were enriched. Interestingly, MapMan (Usadel et al., 2009) analysis (Supplementary Figure 1B) also revealed the induction of Dof (DNA-binding with one finger) transcription factors that were previously described to be involved in biotic stress response, synthesis of seed storage proteins, seed development, photosynthetic processes, and flowering (Wen et al., 2016). For downregulated genes in the 2′,3′-cAMP-treated samples, we observed overrepresentation of processes involved in chloroplast organization (4,9) and protein folding (3,9) and, interestingly, many heat shock proteins and genes involved in the abiotic stress response (Supplementary Figure 1A). For adenosine, immune response and defense regulating pathways, indole-containing compound catabolic processes, and response to hypoxia were highly enriched (Figure 2E, Supplementary Table 6). Interestingly, comparison between 2′,3′-cAMP upregulated genes and adenosine downregulated genes revealed an overlap of 65 genes, mostly involved in stress response, precisely in response to wounding and JA (Figure 2F, G, Supplementary Table 7). This suggests that 2′,3′-cAMP and adenosine might have an antagonistic function in the cell in response to stress, such as wounding, but a synergistic function in the cytokinin-activated signaling pathway (upregulation), transport of transcription factors to the nucleus (upregulation), and response to heat (downregulation). However, general response, looking at the very small overlap is rather independent. After 6 h, the response to both 2′,3′-cAMP and adenosine compound was quite strong. Among the main processes that were upregulated under 2′,3′-cAMP treatment were photosynthesis, hormonal response, and stress response, while genes encoding for response to oxidative stress and metabolism biosynthesis of different compounds were the main ones to be induced (Supplementary Table S8). The main processes that were repressed after 6 h of the 2′,3′-cAMP treatment were related to the RNA machinery and processing, which again supports the stress-related response (Supplementary Table 9). After 6 h of adenosine treatment, the genes involved in the regulation of the hormonal response to gibberellin and hormonal transport were

downregulated. Overall, we observed different responses at the transcriptomic level between 2′,3′-cAMP and adenosine. Therefore, we can further focus only on the response to 2′,3′-cAMP.

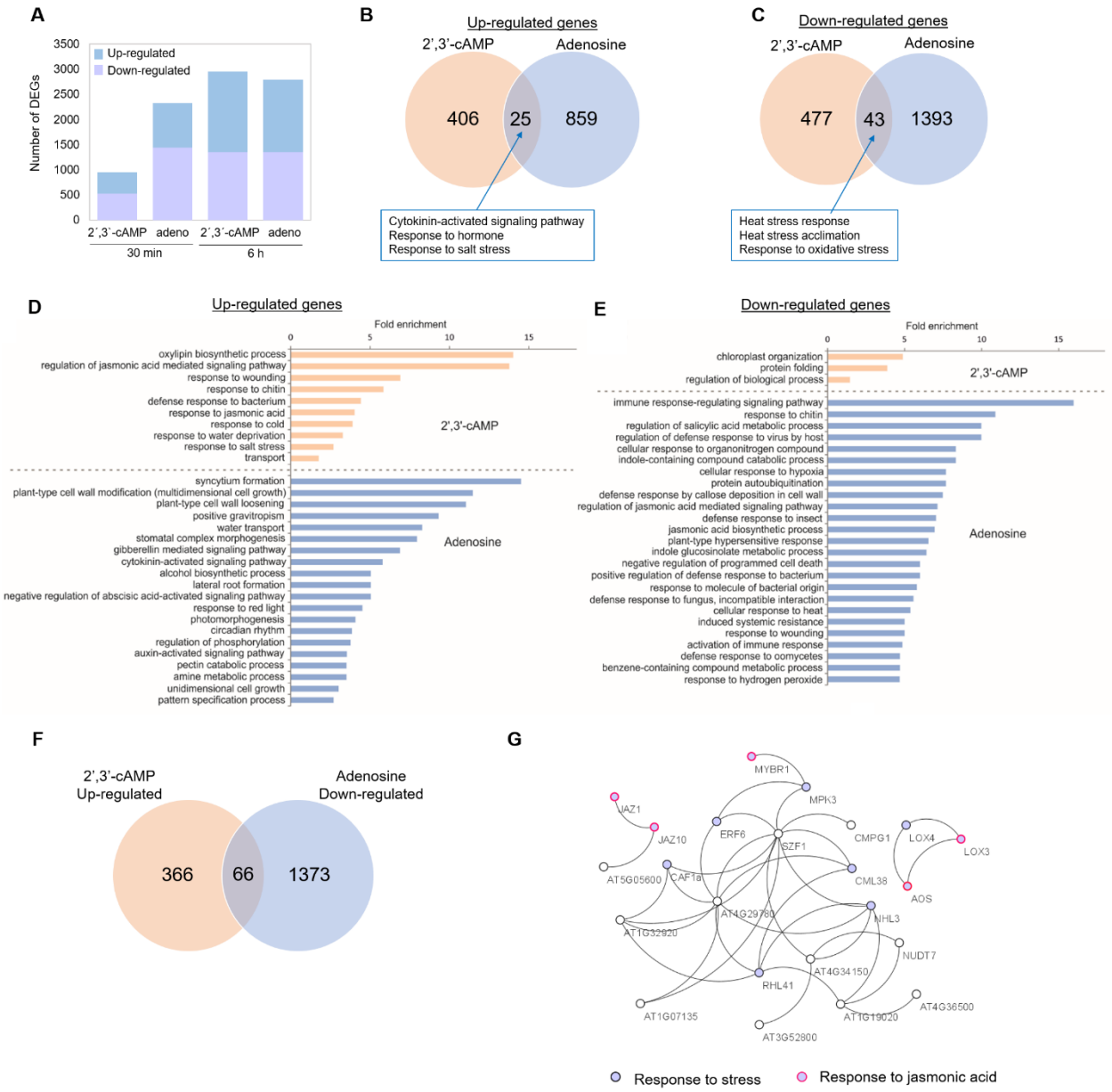

Figure 2. Differential gene expression analysis revealed antagonistic behavior of 2′,3′-cAMP and Adenosine. **A.** The number of genes found to be upregulated or downregulated after 30 min and 6 h of treatment. **B.** Venn diagram representing data summary of all significantly upregulated genes after 30 min of 2′,3′-cAMP and adenosine treatment. **C.** Venn diagram representing data summary of all significantly downregulated genes after 30 min of 2′,3′-cAMP and adenosine treatment. The numbers (**B, C**) correspond to DEGs identified as significantly changed compared to the control

samples in each experiment. **D.** Graph represents the enriched biological process in the dataset of upregulated, specific genes for 2′,3′-cAMP (orange bars) and adenosine experiment (blue bars). **E.** Overrepresentation of the biological process in a set of downregulated, specific genes in 2′,3′-cAMP (orange bars) and adenosine experiments (blue bars). D–E. Overrepresentation is shown as a significant fold enrichment based on the PANTHER Overrepresentation Test (Mi et al., 2017), using Fisher's exact test with FDR multiple correction (p-value ≤ 0.05) and Arabidopsis thaliana as reference organism. **F.** Venn diagram showing an overlap between downregulated genes in adenosine treatment and upregulated genes in 2′,3′-cAMP treatment. **G.** Network of enriched genes was retrieved by the STRING database (Szklarczyk et al., 2017). Experimental evidence, databases, and a low confidence cut-off were used to visualize protein–protein interactions. Genes encoding for proteins involved in stress (violet) and response to JA (violet with pink circle) are highlighted.

2′,3′-cAMP affects proteins involved in abiotic stress response and metabolism

To gain more insight into the response to 2′,3′-cAMP treatment at the proteome level, we analyzed the changes in the abundance of proteins at all five time points. After stringent analysis, the abundance of 472 proteins was significantly changed due to the 2′,3′-cAMP treatment (Supplementary Table S1) (ANOVA, *p-value* FDR corrected ≤ 0.05). Analysis of the localization frequency of significantly changed proteins revealed that the majority of upregulated proteins are localized in the plastid, cytosol, mitochondrion, and nucleus (Figure 3A). Frequency of the same cellular localization is differently distributed between upregulated and downregulated proteins; for example, a higher percentage of nuclear proteins were downregulated, while more plastidial proteins were upregulated, suggesting that different processes might be downregulated or upregulated. Indeed, functional analysis revealed that upregulated proteins are involved in amino-acid biosynthetic processes, auxin transport, photosynthesis, or enzymatic reactions such oxidation–reduction. Interestingly, downregulated proteins belong to the group of proteins regulating protein folding, energy metabolism, response to heat, and translation (Figure 3B). These are usual processes that are affected by stress, therefore observed changes induced by 2′,3′-cAMP resemble stress response. Recently, it has been demonstrated that 2′,3′-cAMP might induce SG formation (Kosmacz et al., 2018). SGs are non-membranous aggregates formed in response to

stress (Sorenson and Bailey-Serres, 2014; Gutierrez-Beltran et al., 2015; Jang et al., 2020) and have been shown to contain proteins from translation machinery, proteins involved in proper protein folding, heat shock factors, and others. Hence, we decided to zoom into proteins known to have a connection with SG formation. Interestingly, among differentially affected proteins, 23 were previously reported to be localized into SGs (Figure 3C, Supplementary Table 1), 12 upregulated and 11 downregulated. Among the most affected SGs proteins, RHM2, RHM1 (Kosmacz et al., 2019), and TSN2 (Gutierrez-Beltran et al., 2015) are known to be crucial components of SGs. That further proves the regulatory connection between 2′,3′-cAMP and stress response at the level of SGs.

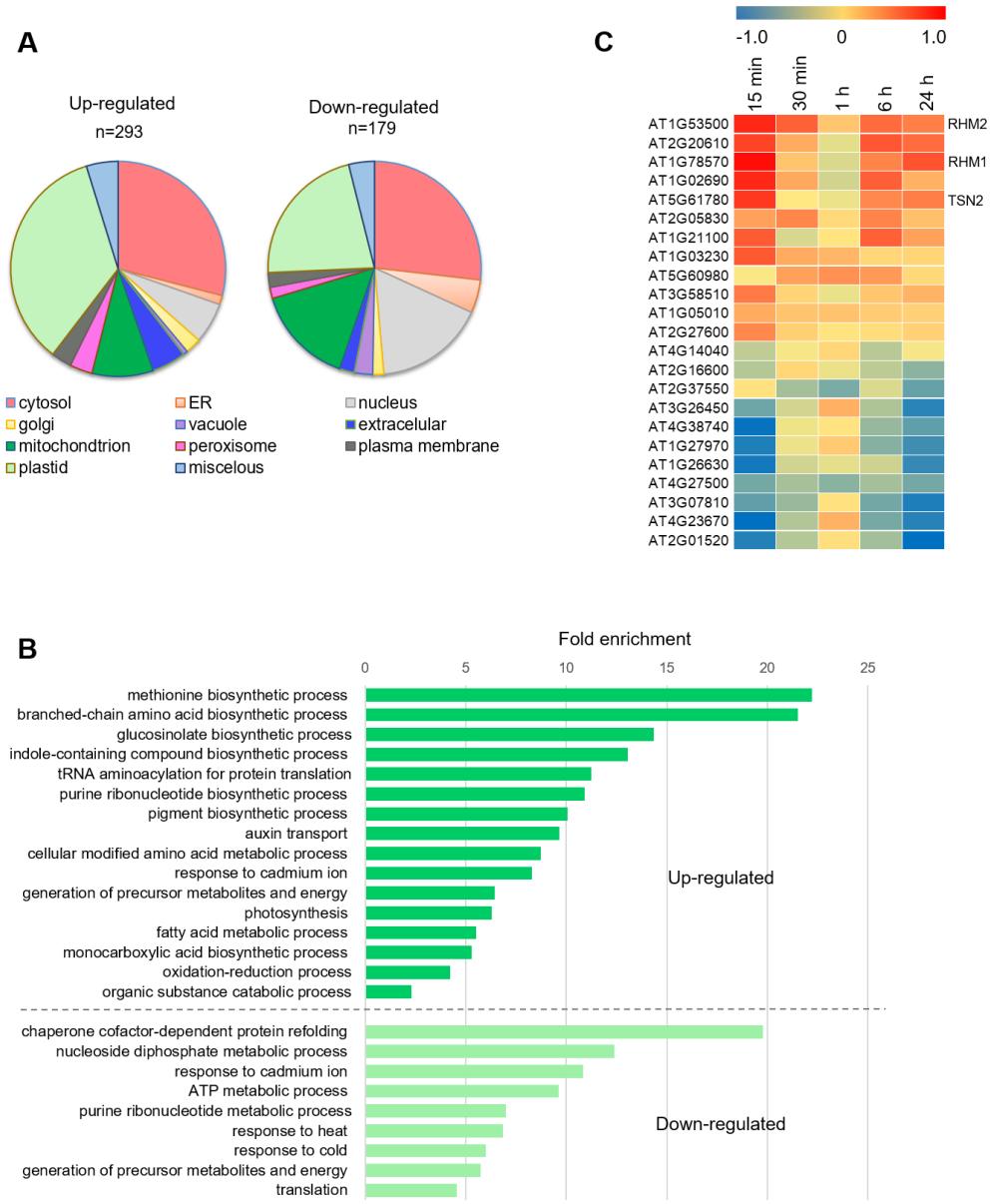

Figure 3. 2′,3′-cAMP induces stress-related changes in the proteome of Arabidopsis thaliana. **A**. Cellular compartment distribution of the identified, significantly upregulated, and downregulated proteins after Br-2′,3′-cAMP treatment. Subcellular localizations for each protein were identified using the SUBA3 database (http://suba3.plantenergy.uwa.edu.au/). **B**. Graph represents enriched biological process in the dataset of significant upregulated (dark green) and downregulated (light green) proteins for 2′,3′-cAMP treatment. Overrepresentation is shown as a significant fold enrichment based on the PANTHER Overrepresentation Test (Mi et al., 2017) using Fisher's exact test with FDR multiple correction (p-value ≤ 0.05) and Arabidopsis thaliana as reference organism.

C. Changes in the abundance of SG proteins are presented as heat maps. Significant changes were determined using one-way ANOVA (p-value ≤ 0.05, n = 4 biological replicates).

2′,3′-cAMP induces movement of PBs

Knowing that SGs are highly connected with PBs and having in mind that 2′,3′-cAMP is closely related to metabolism of RNA (being its degradation product), it would be crucial to evaluate whether 2′,3′-cAMP can also affect PBs. The main function of PBs is translational repression and mRNA decay (Kedersha et al., 2005; Xu and Chua, 2011). However both PBs and SGs were shown to have continuous interaction (Anderson and Kedersha, 2009) and often be transiently linked to each other (Eisinger-Mathason et al., 2008; Buchan et al., 2012). Unlike SGs, PBs are always present in the cell, independently from stress conditions; however, stress might affect the dynamic of PBs. To evaluate this, we decided to focus on DCP1-GFP protein (a well-known PB marker) and determine whether treatment affects PB localization. Using confocal microscopy and aligning t-stags (Figure 4A, B), we followed the movement of PBs over time. Particle tracking showed that the application of 2′,3′-cAMP significantly induced not only displacement length (Figure 4C, Supplementary Table 10) but also speed of PB movement (Figure 4D) compared to the control (Supplementary Table S11).

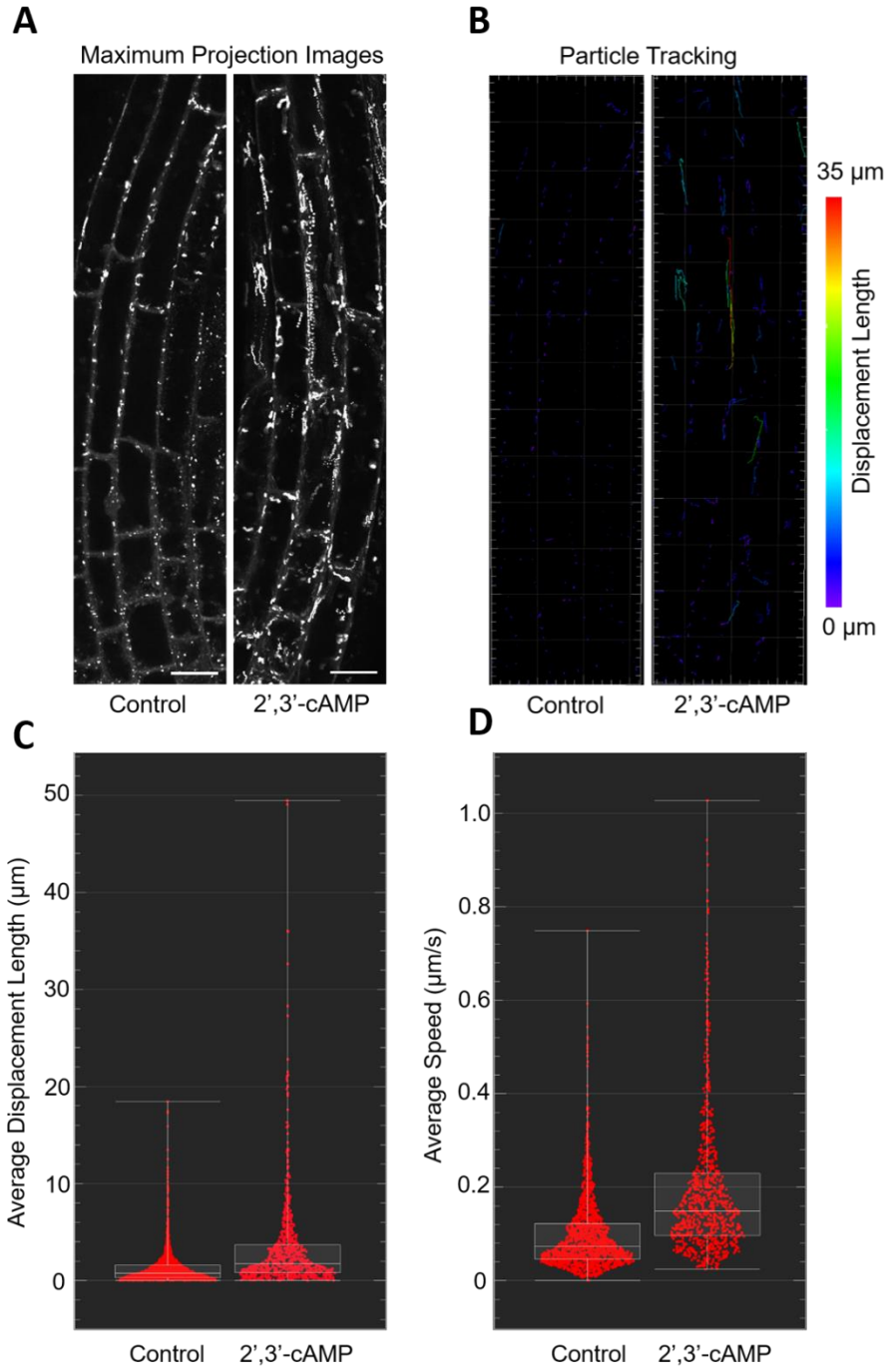

Figure 4. 2′,3′-cAMP induces movement of PBs. A. Maximum projection images from 61 time points collected from DCP1-GFP Arabidopsis seedlings under control and 2′,3′-cAMP treatment. **B.** Particle tracking in the control cells and 2′,3′-cAMP-treated cells. Scale represents the color code for the distance of displacement for each PB. **C.** The average displacement length of the PB particles in the cell is expressed in µm. **D.** The average speed of PB movement in control and 2′,3′-

cAMP-treated seedlings. Speed is expressed in µm/s. For **C** and **D**, control n = 1583, 2′,3′-cAMP n = 898.

Discussion

As 2′,3′-cAMP has been detected to be present in plants (Pabst et al., 2010), proven to respond to stress conditions (Van Damme et al., 2014), and reported as a facilitator of stress granule formation (Kosmacz et al., 2018), the next step is to understand its general function in plant cell response at different molecular levels. We believe that this is the first study that explores the comparison of cellular responses in plants at the metabolic, proteomic, and transcriptomic levels after the application of exogenous, membrane permeable 2′,3′-cAMP (Br-2′,3′-cAMP). Combination of omics approach (Moreno et al., 2021) was shown to be a valuable tool for uncovering the biological function therefore we decided to use it for 2′,3′-cAMP. Since, in animal cells, 2′,3′-cAMP has been shown to be metabolized to adenosine and LC/MS data analysis revealed that in plants, after 30 min of treatment, there is a decrease in Br-2′,3′-cAMP and an increase in Br-adenosine (Figure 1A), we first evaluated transcriptional response to 2′,3′-cAMP and adenosine (Br-adenosine).

Transcriptomic data revealed that, although there was an overlap between genes that were upregulated by 2′3,′-cAMP and downregulated by adenosine, suggesting that both molecules, at least in some processes, might play an antagonistic role, the remaining changes were significantly different, supporting the rather distinct function of 2′,3′-cAMP and adenosine. At the 30 min time point, the 2′,3′-cAMP-induced genes were involved in many biological processes, with the highest fold enrichment for genes responding to biotic and abiotic stress, such as heat shock factors, transcription factors, and response to JA. This parallels the metabolomics data, where the accumulation of mainly RNA-degradation products and dipeptides (29) (Figure 1C) are signatures for stress response (Doppler et al., 2019; Thirumalaikumar et al., 2020; Camilo Moreno et al., 2021) not only in plants (Luzarowski et al., 2021).

Interestingly, many of the affected processes at the transcript plant (RNAseq) and metabolic levels were also reflected at the proteome level. For example, many proteins involved in response to abiotic stress, such as cold or heat stress, were downregulated in response to 2′,3′-cAMP. Also, translation machinery was inhibited by the treatment. It has been shown that 2′,3′-cAMP can bind to RNA-binding motives in the protein sequence of Rbp47b, an SG marker to facilitate SG formation (Kosmacz et al., 2018). Interestingly, it was further confirmed by small molecule-

protein complex studies that seven out of the 31 high-confidence 2′, 3′-cAMP targets predicted by SLIMP (Zuhlke et al., 2021) contain an RRM domain. The identified list also contains a plastidial protein CP29, a component of the plastidial SG (Chodasiewicz et al., 2020). Following that, our proteomic data revealed that the key components of SGs (RHMs and TSN, among others) were over-accumulated under treatment with 2′,3′-cAMP. Adding to SGs, using confocal microscopy, we further showed that 2′,3′-cAMP increases the dynamic of PBs, which are known to interact closely with the component of SGs. All of this again confirms the involvement of 2′,3′-cAMP in the regulation of stress response.

Overall, our results indicate that 2′,3′-cAMP can not only trigger response at metabolite, protein, and transcript levels but can also trigger changes already in the first 15–30 min of the treatment. One would expect that, as 2′,3′-cAMP is metabolized to adenosine in plant cells, both molecules might share a common response, but except for an overlap in a particular niche of response that seems to be antagonistic triggering changes at metabolic and transcriptomic levels, each of the molecules triggers a rather specific response. As already suggested in the literature, but finally experimentally proven, 2′,3′-cAMP causes changes characteristic of abiotic stress response, such as the accumulation of dipeptides and RNA-degradation products or the accumulation of proteins/transcripts involved in stress response or stress regulation.

Material and methods

Plant growth conditions and feeding experiment

Arabiodopsis thaliana Col-0 seedlings were grown in liquid medium (Murashige and Skoog, 1962) supplied with 1% sucrose in continuous light. After seven days, the medium was exchanged, and after three days (at 10th day), treatment with 1 µM Br-2′,3′-cAMP was performed. As a control, the seedlings were treated with water (in which the compound was dissolved). Next, seedlings were harvested after 15 min, 30 min, 1 h, 6 h, and 24 h of treatment, quickly dried on paper, and freezed in liquid nitrogen. Three days of lyophilization were used to obtain dry plant material. Experiment using the same conditions with 1 µM Br-adenosine was performed for transcriptome analysis.

Metabolite and protein extraction

The protocol for the extraction of molecules was adjusted from (Salem et al., 2017). Using 10 mg of dried tissue powder, macromolecules were extracted using a methyl tert-butyl ether (MTBE)/methanol/water solvent system, which separates molecules into pellet (proteins), organic (lipids), and aqueous phases (primary and secondary metabolites). Equal volumes of particular fractions were dried using a centrifugal evaporator and stored at –80°C before metabolomic analysis.

LC–MS secondary metabolomics

The dried aqueous phase was measured using ultra-performance liquid chromatography coupled to an exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) in positive and negative ionization modes, as described in (Giavalisco et al., 2011). Processing of data was performed using REFINER MS 10.5 (GeneData; http://www.genedata.com) and included peak detection, chemical noise subtraction, retention-time (RT) alignment, and integration of isotopic peaks into peak clusters. Metabolite features were annotated using an in-house reference compound library allowing 10 ppm m/z and 0.1 min RT deviations.

LC–MS/MS for proteins and data analysis

Protein pellets formed in the MTBE-based extraction method were solubilized in 100 µl of urea–thiourea buffer (6 M urea, 2 M thiourea in 40 mM ammonium bicarbonate). Protein content was determined using a Bradford assay (Carl Roth, Karlsruhe, Germany). 40 µg of protein was treated with 5 mM of dithiothreitol (DTT) for 30 min at RT followed by cysteine alkylation with 15 mM iodoacetamide for 20 min at RT in the dark. Next, enzymatic digestion of proteins using LysC/Trypsin Mix (Promega, Fitchburg, WI) was performed according to the technical manual. After digestion, samples were acidified with trifluoroacetic acid (TFA) to pH < 2. Peptides were desalted using C18 Empore® extraction discs (3M, Maplewood, MN) STAGE tips (Rappsilber et al., 2003) and dried to approximately 4 µl using a centrifugal evaporator. Samples were stored at –80°C until measurement. Dried peptides were solubilized in loading buffer (2% ACN, 0.2% TFA), and an equivalent of 0.8–1.0 µg of peptides was separated using a reversed-phase column and analyzed on a Q-Exactive Plus or Q-Exactive HF spectrometer (Thermo Fisher Scientific). MaxQuant version 1.6.0.16 (Cox and Mann, 2008) and its build-in search engine Andromeda (Cox et al., 2011) were used to analyze the raw proteomic data. For protein annotation, the *A. thaliana*

protein database, from 2014 and last updated in December 2017, was used. Search included also a contaminant database. Contaminants and decoy hits were removed from each dataset. Furthermore, at least two unique peptides were required per protein group. Label free quantification (LFQ) intensities were used in all analysis performed in this manuscript.

Analysis of the protein and metabolite data from the feeding experiment.

GeneData derived raw metabolite intensities were normalised to the median intensity of all mass features detected in a given chromatogram. MaxQuant derived LFQ intensities were used for further analysis. Both metabolite and protein data were subjected to $\log_2$ transformation prior two-way analysis of variance (ANOVA) analysis implemented in MeV software (Howe et al., 2011) using treatment (treated versus untreated) and time (15 min, 30 min, 1 h, 6 h, 24 h) as variables. Obtained p-values were subjected to FDR correction to select metabolites and proteins significantly affected by the treatment. Software MeV version 4.9 (Saeed et al., 2003) was used to obtain heat maps.

RNA extraction and RNAseq analysis

An RNA extraction kit (Macherey-Negel) was used to extract total RNA from 10 mg of lyophilized tissue, followed by quality assessment by a Bioanalyzer RNA 6000 nano (Agilent). RNAseq analysis was performed by Lexogen GmbH using QuantSeq 3′-mRNA library preparation and QuantSeq 3′-UTR NextSeq SR75 sequencing. To perform RNAseq analysis, three biological replicates for two time points of 30 min and 6 h were used in each experiment. MapMan software (Usadel et al., 2009) was used to visualize perturbations in gene expression.

Processing body dynamic assessment under confocal microscope

Arabidopsis seeds expressing GFP-tagged DCP1 (Decapping protein 1), the PB marker, were kindly provided by Dr. Emilio Gutierrez-Beltran. Plants were grown for 5–7 days on MS media supplied with 1% of sucrose. At the day of the experiment, seedlings were moved to an Eppendorf tube and incubated either with water (control) or with 100 µM Br-2′,3′-cAMP for 30 minutes. After incubation, seedlings were observed under a confocal microscope (Leica TCS SP8) using x,y,t function. GFP was excited using 488nm laser and emission was obtained between 500nm to 600nm. Images were collected every two seconds for a total of 69 images, which were used for

video assembly. The calculations of PB displacement and speed of displacement were done using IMARIS software (https://imaris.oxinst.com/).

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