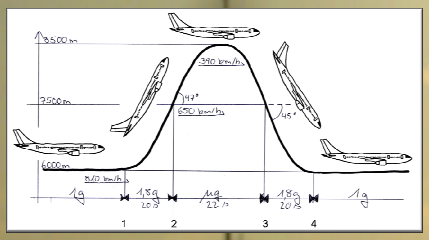
GENE EXPRESSION OF SELECTED WRKY TRANSCRIPTION FACTORS IN *ARABIDOPSIS THALIANA* CELL CULTURES DURING A PARABOLIC FLIGHT

Anne Hennig, Maren Neef, Niklas Hausmann, Rüdiger Hampp

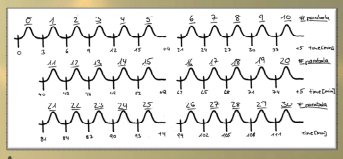
University of Tübingen, Physiological Ecology of Plants, Auf der Morgenstelle 1, 72076 Tübingen (Germany)

EBERHARD KARLS UNIVERSITÄT TÜBINGEN

Plants use gravity as a guide for growth and development. Recent studies demonstrate that plant cells which are not part of specialized tissues such as the root columella cells are also able to sense gravitational forces. Therefore we used undifferentiated, homogeneous cell cultures of *Arabidopsis thaliana* (cv. Columbia) (A.t.) in order to identify early alterations in gene expression in response to altered gravitational fields. As experimental system we used parabolic flights (A300, Novespace). A single parabola consists of following phases: 1 *g*, 1.8 *g* for 20 sec, microgravity (µ *g*) for 22 sec, 1.8 *g* for 20 sec, 1 *g*. Up to 31 parabolas are executed within one flight. By the use of microarrays (ATH1, Affymetrix) we found several hundred transcripts to be altered in amount during the respective gravity segments. For this study, we specifically looked for genes at the beginning of signal transduction chains, such as those coding for transcription factors (TFs). TFs are small proteins regulating gene expression of their target genes by binding to specific promoter sequences. For quantification via qRT-PCR, six WRKY genes were selected. WRKYs are known to be involved in various physiological processes such as senescence or pathogen defense. The data indicate a transient gene expression profile, while most of the analyzed WRKYs were significantly down-regulated after the microgravity-phase of the parabola compared to the 1 *g* control.



Schematic view of a parabolic flight maneuver. When the plane reaches an altitude of about 6100 m and a speed of approximately 810 km/h, the A300 goes into a steep ascent (1). Due to this climb flight, the *g*-force increases from regular 1 *g* to 1.8 *g*. This hypergravity condition lasts for 20 sec, until the angle of ascent arrives at 47° (2). At this point, the engines were shut downn to a very low thrust. Within a few sec, the aircraft goes into free fall. The speed it has gathered before keeps the Airbus moving upwards till the apex of the curve. The A300 slows down to 390 km/h. From here, it continues to descend in an open parabola (22 sec). At the end of the microgravity phase (3), when the plane reaches a downward angle of 42°, the A300 is pulled out of the dive and back to the horizontal position (1.8 *g* 4). Altogether 31 parabolas were flown within one flight day.



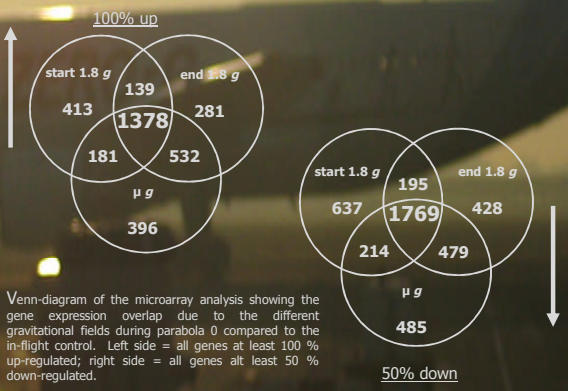
A typical parabolic flight sequence with 31 parabolas lasts approximately 2 h – 2 h 30 min. Series of five (respectively six) flown parabolas were interrupted with 4 – 8 min 1 *g* intervals.

A.t. cell suspension culture cultivation

Syringe-based flight hardware

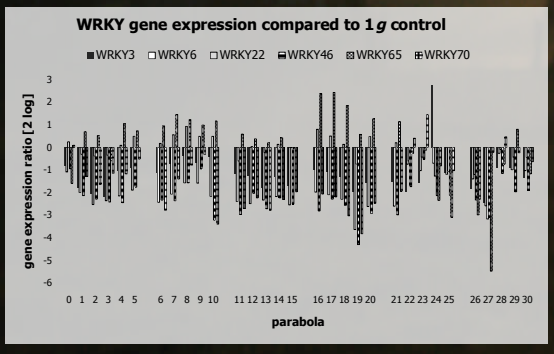
Manual fixation of A.t. cell cultures

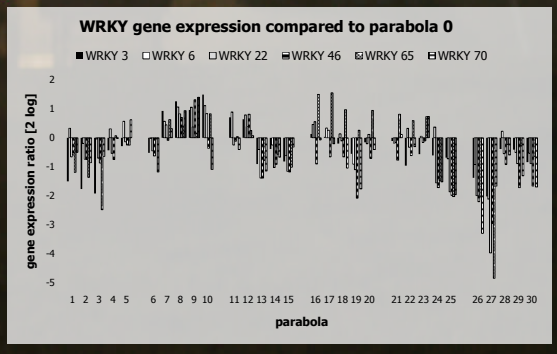
For gene expression analysis, wild type A.t. cell cultures were manually fixed by piston actuated closure of syringes, filled with RNAlater (Ambion) at the end of every different gravitational phase during the first parabola. After the microarray (ATH1/Affymetrix) screening, we observed specific gene expression profiles due to the different *g*-forces, although the main part of the significantly up- or down-regulated transcripts responded to each treatment, indicating a general stress response. Interestingly, we found also genes, which were specifically expressed after a certain *g*-force. E.g. 396 transcripts were at least 100% up-regulated during the microgravity-phase of the parabola.



Venn-diagram of the microarray analysis showing the gene expression overlap due to the different gravitational fields during parabola 0 compared to the in-flight control. Left side = all genes at least 100 % up-regulated; right side = all genes alt least 50 % down-regulated.

Total genome analysis via microarrays





WRKY gene expression quantification

Since only 34 out of 76 WRKY genes are present on the microarray, we decided to precisely analyze expression changes of six selected WRKY TFs via qRT PCR (IQ5; iScript one step with SYBR Green/BioRad). WRKYs can be divided into three distinct groups out of which at least one gene per category was used for this study. Only total RNA samples taken after the microgravity-phase of each parabola were analyzed. Alterations in the transcript amount were determined as described by Pfaffl et al. (REST-384/Nucl. Acids Res. 2002). With few exceptions (e.g. WRKY 65) all investigated WRKYs exhibited a transient, down-regulated expression profile compared to the 1 *g* control. However, if the first parabola (0) serves as control, the gene expression trend over the parabolic flight changes entirely. Here, we observed only at the beginning and at the end of the flight a constant down-regulation, whereas the gene expression in between showed no specific pattern.

Significant gene expression changes in undifferentiated plant cells occur within a very short time (sec) of exposure during a parabolic flight. In future studies we will analyze more WRKY members via qRT-PCR, comprising possible target genes of this TF-family. Additionally, more samples will be taken during the next parabolic flight campaign.

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