To elucidate the mechanism by which REV1 promotes DNA damage bypass, we have analyzed the progression of replication on ultraviolet light-damaged DNA in mouse embryonic fibroblasts that contain a defined deletion in the N-terminal BRCT domain of REV1, or that are deficient for REV1.

DNA fiber labeling method that has been previously described has been adapted in which two modified nucleotides IdU and BrdU were used to label newly replicated DNA. Incorporated IdU and BrdU were detected by fluorescent immunolabeling and the progression of replication fork was monitored. To examine the effect of UV damage to DNA on replication fork progression, cells were treated with either 20J/m^2 or 40J/m^2 UV dose at the end of first labeling period (IdU) and before second labeling (BrdU). Fork rates were calculated for each labeling period and the ratio of IdU to BrdU were analyzed. Under normal replication conditions ratio of IdU to BrdU is approximately 1. However an increase in this ratio directly corresponds to the rate of fork stalling during second labeling as a result of UV damage to DNA.

To investigate the role of REV1 BRCT and REV1 in replication fork progression, DNA fiber spreads were prepared, labeled forks were measured and compared to that of wild type cell line. In wild type cells with no UV treatment the average ratio of IdU to BrdU was 1.13 and REV1 BRCT and REV1 mutant cells showed an average ratio of 1.13 and 1.16 respectively. There was no significant difference in the rate of fork progression in any of the mutant cell lines as compared to the wild type line. Therefore, these genes are dispensable for the normal growth and viability of the cell.

The frequency of fork stalling in REV1 BRCT and REV1 mutant cell lines after 20J/m^2 or 40J/m^2 UV dose was measured. Both REV1 BRCT and REV1 mutant cells showed a significant increase in the ratio of IdU to BrdU in response to 20J/m^2 UV treatment and the ratios increased further at 40J/m^2 UV. In wild type cells with 20J/m^2 and 40J/m^2 UV treatment the average ratio of IdU to BrdU increased from 1.13 to 1.92 and 3.24. REV1 BRCT and REV1 mutant cells showed an increase in the ratio from1.13 to 4.14 and 4.99, 1.16 to 3.43 and 4.5 respectively. Furthermore, the exogenous expression of mREV1 in REV1 BRCT and REV1 mutant cell lines restored wild type phenotypes.

These results provide an evidence for the role of BRCT domain of REV1 in response to DNA damage and that REV1 plays a central role in replication fork progression of UV-damaged DNA.

Jansen J, Langerak P, Tsaalbi-Shtylik A, van den Berk PC, Jacobs H, de Wind N (2006) Strand-biased defect in C/G transversions in hypermutating immunoglobulin genes in Rev1-deficient mice. J Exp Med 203:319-323.
Jansen JG, Fousteri MI, de Wind N (2007) Send in the clamps: control of DNA translesion synthesis in eukaryotes. Mol Cell 28:522-529.
Merrick CJ, Jackson D, Diffley JF (2004) Visualization of Altered Replication Dynamics after DNA Damage in Human Cells. J Biol Chem 279(19):20067-20075.

Supervisor: Haracska Lajos
E-mail : hima@brc.hu

Uptake and degradation of xenobiotic in *Sphingomonas subarctica* SA1

Anna Gara

Department of Biotechnology, University of Szeged, Szeged, Hungary

Sulfanilic acid is a typical representative of sulfonated aromatic amines widely used and manufactured as an important intermediate in the production of azo dyes, plant protectives and pharmaceuticals. *Sphingomonas subarctica* SA1, a Gram-negative aerob bacterium is able to utilize sulfanilic acid as the only carbon, nitrogen, and sulfur source (Perei et al. 2001). In addition to sulfanilic acid, *Sphingomonas subarctica* SA1 could degrade six other aromatic compounds, such as sulfocatechol, protocatechol, para-amino benzoic acid, 3,5-dihydroxybenzoic acid and oil in soils.

Comparison of the protein patterns of cells grown on various substrates revealed that the strain used alternative metabolic pathways for biodegradation of these compounds. However, similar patterns were observed in the case of cells grown on sulfanilic acid and sulfocatechol. Therefore sulfocatechol is supposed to be formed, as first intermediate in the catabolism of sulfanilic acid. Unfortunately, sulfanilic acid could be converted by intact cells only but not by disrupted cells, thus the characterization of this reaction step was difficult. Nevertheless, sulfocatechol is further oxidized by a ring cleaving dioxygenase, named as sulfocatechol dioxygenase. This enzyme was partially purified and identified by mass spectrometry (Magony et al. 2007). A genomic locus harbouring the genes of sulfocatechol dioxygenase (*scaEF*) was also identified and upstream from these genes, few other *orfs* coding for proteins similar to muconate cycloisomerases (ScaA), lactone hydrolases (ScaB), maleylacetate reductases (ScaC) and an oxidase (ScaD) were recognized. These enzymes were actively overexpressed in *E. coli* and the sulfocatechol degradation pathway was reconstituted by the recombinant proteins.

The first step of sulfanilic acid degradation is not fully understood. The enzymes probably converting sulfanilic acid to sulfocatechol were very sensitive to cell disruption indicating that they were somehow related to the membrane. Proteomics approach was applied to identify of the enzymes catalyzing the sulfanilic acid conversion. Bands sepcifically appearing upon substrate induction in the membrane and soluble fractions were cut out and sequenced *de novo* by mass spectrometry.

The analysis of the proteomic data of the soluble fraction led to the identification of another gene set in the genome. In this locus, two genes likely coding for proteins involved in the oxidative deamination of sulfanilic were predicted.

Three specifically appearing membrane proteins were found in the membrane fractions of cells grown on sulfanilic acid. The aminotransferase is probably one component of the sulfanilic acid converting enzymes catalyzing the deamination of sulfanilic acid. It was shown to be poorly membrane associated, since it was also found in the soluble fractions. The second protein contained motifs of ATP-binding casettes indicating the energy-dependence of sulfanilic acid uptake. The third protein is a hypothetical TonB-dependent protein, which might play a role in many types of transport including iron uptake. The expression of the TonB-dependent protein is upregulated specifically by xenobiotics/aromatics and iron. Since, two enzymes of the degradation pathway are known to contain iron in their active center, it is plausible to assume, that the TonB dependent protein is involved in the iron transport to feed the extra iron demand of the enzymes taking part in the biodegradation.

From our data it is assumed that the uptake and conversion of sulfanilic acid is linked to a membrane protein complex and this association can function as a self-defending mechanism for the cell against the cytoplasmic occurrence of the toxic substrates. Furthermore, a potential link between the xenobiotics degradation and iron transport is suggested.

Magony M, Kákonyi I, Gara A, Rapali P, Perei K, Kovács KL, Rákhely G (2007) Overlaps between the various biodegradation pathways in Sphingomonas subarctica SA1. Acta Biol Hung 58:37-49.
Perei K, Rákhely G, Kiss I, Polyák B, Kovács KL (2001) Biodegradation of sulfanilic acid by Pseudomonas paucimobilis. Appl Microbiol Biotechnol 55:101-107.

Supervisor: Gábor Rákhely
E-mail: anna.gara@gmail.com

Role of salicylic acid pretreatment on the photosynthetic performance and the generation of reactive oxigen species and nitric oxide in tomato plants (*Solanum lycopersicum* Mill. L. cvar. Rio Fuego) under salt stress: acclimatization or programmed cell death

Katalin Gémes

Department of Plant Biology, University of Szeged, Szeged, Hungary

Salicylic acid (SA) has long been known as a signal molecule in the induction of defense mechanisms in plants (Raskin 1992) and it was shown to improve the acclimation to different abiotic stress factors, including high salinity (Szepesi et al. 2009). SA increased reactive oxygen species (ROS) production and therefore oxidative stress (Knörzer et al. 1999). SA was also shown to influence a number of physiological processes (Raskin 1992) e. g. inhibited the activity of Rubisco and thus the photosynthetic activity (Vernooij et al. 1994).

The aim of my work was to reveal, how ROS production ($O^{\cdot}$; H_2O_2) was modified by different concentrations of SA, how the SA-treated cells could acclimate to oxidative stress or why other tissues became committed to programmed cell death (PCD).

On the basis of the inhibity effect of SA on stomatal conductance and photosynthetic performance, which has been documented in several papers, it was presumed, that the ROS produced after SA pretreatment may be derived from an inhibited photosynthetic electron transport. This may also reduce the plants capacity to synthesise compatible osmolytes, such as sugars, during pretreatment or salt stress.

That is why we measured the changes in photosynthetic activity (chl*a* fluorescence induction parameters, CO_2 fixation rate as function of PAR or Ci and stomatal conductance) during pretreatment.

Tomato plants were grown hydroponically in the presence of different SA (10^{-3} M, 10^{-4} M, 10^{-7} M). Seven-week-old plants were exposed to 100 mM NaCl for a week.

Short-term pretreatment of plants with 10^{-3} M SA resulted in a permanent decrease in the stomatal conductivity and the CO_2 fixation rate compared to the control and also decreased the viability of plants. In contrast, after a transient decline photosynthetic parameters of plants grown in 10^{-7} and 10^{-4} M SA were not significantly different from the untreated control at the end of the pretreatment period. Salt stress also inhibited the photosynthetic activity, which was significantly alleviated by 10^{-4} M SA. The improved photosynthetic performance and the accumulation of soluble sugars as compatible osmolytes resulted in a partial osmotic adjustment and contributed to successful acclimation to high salinity in 10^{-4} M SA pretreated plants.

The accumulation of putrescine in the leaves and those of spermidine and spermine in the roots are adaptive feature of some halophyte species. We found similar changes in the polyamine spectrum of plants grown in 10^{-4} M SA at the end of pre-treatment period. Moreover these tissues produced less ethylene, a PCD inducing plant hormone, which coincided with higher viability of root apical cells.

As it was expected a significant accumulation of H_2O_2 occurred in the leaves and roots of plants exposed to 10^{-4}-10^{-7} M SA, but after three weeks the differences disappeared in the root tissues and remained in the leaves. We prepared mesophyll protoplasts as model system to investigate the effects of the compounds that accumulated in plants during pre-treatments on ROS production and to compare the results with intact plants.

Knörzer OC., Lederer B, Durner J, Böger P (1999) Antioxidative defense activation in soybean cells. Physiol Plant 107:294-302.
Raskin I (1992) Role of salicylic acid in plants. Ann Rev Plant Physiol And Plant Mol Biol 43:439-463.
Szepesi Á, Csiszár J, Gémes K, Horváth E, Horváth F, Simon LM, Tari I (2009) Salicylic acid improves the acclimation to salt stress by stimulating abscisic aldehyde