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Ph.D. program for Biology
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Examination of stress processes caused by chromium compounds and viral proteins on *Schizosaccharomyces pombe* cells

Ph.D. thesis

Judit Antal

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INTRODUCTION

The homeostasis reveals the dinamic balance, allows to the living systems the self-supporting operations and also to be able to buffer the impacts of external changes as well. Attacts come not only from the extralell world but in the intracellular space happen because of the outcome of respiratory processes or some by-products of enzymatic reactions and so for example reactive oxigene species (ROS) are able to act as harmful agents to the cells.

Some elements including special metal compounds are essential for living organisms to exist. In healthy environment metals usually represented as trace elements, enzymatic contents in nanomolar concentration. Widely used in industry there are some special toxic compounds containing the unstabile, reactive Cr^{VI}. The reduction of this chromium ion happens rapidly, generating reactive intermediers which are capable to induce wounding intracellular reactions such as inhibition of DNA repair enzymes or suppression of signal transduction pathways. Certain stress from the extracellular world so can be responsible for genotixicity and malignus transformation. Nevertheless there are well-characterized microorganisms resistant to the harmful effects of heavy metals (Nies 1999).

Most of the genetic background mechanisms impacked by the oxidative stress from different sources still remains unknown. The idea of creating and investigating mutant strains was to characterize chromate-sensitivity and –tolerance in fission yeast. To learn more about mutant genes and gene products can be useful to determine which genes are affected and how are they involved in the mechanisms induced by oxidative stressors, heavy metals for example chromium in living cells (Halliwell and Gutteridge, 1999).

Viral proteins are mostly responsoble for the effective infection and also act as stress factors for the organisms attacked by the virus. The HIV-1 virus (human immunodeficiency virus type 1) infection often leads to AIDS (acquired immune deficiency syndrome) encodes some of accessory proteins which possibly play some kind of role in viral infection. This virus also bears a 15 kDa virion associated protein Vpr (viral protein type R) (Elder et al., 2002).

Vpr protein has been shown to be responsible for the effectiveness of the viral infection, helps viral replication cycle and acting on several cell contents enhances viral pahtogenesis. Some of the earlier reports say the Vpr is able to cause oxidative stress in host cells, but no data has been shown about the connetcion between Vpr and oxidative stress or Vpr-expressing *S. pombe* cltures. Some stages of viral infection commonly generate oxidative stress in host cells that is why the therapy ot the AIDS patients contains antioxidant therapy commonly known as coctail (Dunable 1998). The question is whether the intracellular stress caused by the Vpr expression and the oxidative stress from the extracellular world are really adds.

The barley yellow dwarf (BYD) virus, a global desease of cereals appears with the primary symptoms of the infection such as retarded growth and yellish color of the plants. This has an enormous economical impact on crop production because of the significant yield loss. The role of the movement protein (MP) during the BYDV desease has not been reported yet. The only way to identify MP as a potential viral pathogenic determinant to study the effects of BYD viral proteins on basic cellular functions especially on plant growth. If MP has impact on cell proliferation or MP inhibits cell division reveals a possible explanation of dwarf plants. Due to inherent technical difficulties in studying the effects of viral proteins in plant cells *in vivo*, a *S. pombe* model system was used to carry out initial functional screening of special gene products and further validate those findings in plant cells. Use of fission yeast as a model organism studying gene activities of high eukaryotes including viral gene functios have been demostrated previously several times, thus making this unicellular organism an efficient genetically tractable system to study plant-related genes.

AIMS

To study the background mechanisms of the oxidative stress from different sources, the aims in details are presented here:

1. For the characterization of chromate-sensitivity and –tolerance we have created special *S. pombe* recombinants. These chromium-sensitive or tolerant mutants were determined by single mutations, bearing a stabile genetic background and carrying *leu1-32* and *ura4-D18* selective markers.
2. To examine the possible interactions between Vpr protein and the oxidative stress using *S. pombe* cell cultures.
3. To show that the MP expression causes cell cycle arrest in fission yeast.
4. To proove MP expresssion leads to hyperphosphorilation of Cdc2 kinase, which molecule's phophporilation status is a key of the mitosis.
5. To determine new details about the target molecules of this viral protein and to find out more about cell cycle arrest caused by MP.
6. To show that MP is a possible viral determinant of the BYDV and with using a fission yeast system answer the question which are the most important cellular functions affected during the viral infection. Basically we have made attempts to find out if MP does contribute to viral infection and is it responsible for the retarded growth of the infected plants or not.

METHODS

The tetrad analysis of *S. pombe* chromium tolerant strains (*chr1-14 T, chr-09 T)* and chromium sensitive strains *(chr-23S, chr-33S)* were carried out. The spore forming ability was examined by using Bürker chamber.

Spore clones obtained from the crosses were tested for chromium tolerance and auxotrophy by using selective mediums.

To learn more about how Vpr acts on eucaryotic cells we have used *in vivo* methods and special *S. pombe* strains.

In order to turn Vpr gene off or on, no-message thiamine promoter type 1 (nmt1) was used. Thiamine content of the medium ensured full gene repression, and if there was no thiamine present wild-type Vpr was expressed. Cell proliferation was measured with heamocytometer and cell morphology of the fission yeast cells were captured using microscopy and camera.

The experiments on acute and adaptive stress were carried out in special H_2O_2 containing mediums.

The DNA contents of the *S. pombe* cells were measured by flow cytometric analysis in nitrogen limited medium. The samples were fixed wiht ethanol, RNAse added. After propidium iodide staining flow cytometric datas were collected in order to obtain the cellular DNA content and cell length.

We have developed a fission yeast model system to study potential effects of BYD viral proteins on basic cellular functios. The BYDV MP gene was cloned and expressed in an inducible fission yeast expression vector under the control of a nmt1 promoter. The vector was transformed into *E. coli* cells, and then the plasmid DNA checked with restriction digestion and PCR reaction. The MP sequence inserted into the vector was compared with the original using a sequencing reaction and analysis.

After checking the sequences of the vectors we have used Electro Cell Manipulator to transform them into *S. pombe* cells. Cell morphologies, the characteristics of cell division, localization of the MP, and specificities of the gene expression were described in details.

In order to compare the growth rate of *S. pombe* cells expressing MP with those cells in which MP gene expression was repressed or totally missing all type of cells were grown in plasmid selective EMM medium with or without thiamine. We applied flourescent and confocal microscopy and camera in order to study the cell morphology. To ascertain the cell length forward scatter analysis and microscopy was used. A statistical Student's t-test ($p<0,0001$) was used for the calculations.

As an *in vivo* reporter GFP was fused to the N terminal end of MP. We used DAPI for observing the nuclear morphology and calcoflour staining in order to perceive the cell wall and septum.

Experiments were carried out in liquid medium to compare the growth rate of *S. pombe* cells experessing MP with those cells in which MP gene exression was suppressed. The number of cells were determined with Bürker chamber. Colony forming ability of the cells was checked on solid EMM medium.

Flow cytometric analysis of the MP-expressing and non-expressing cultures were followed up on cell-cycle profile and the DNA amount of cells. The cultures have been synchronized in G_1 cell phase. The comparative analysis of the profiles of the MP-expressing and Mp-repressing cells let us to presume the effects of MP on cell cycle.

The phosphorylation status of Cdc2 kinase was measured using western blot analysis. The protein concentrations were determined by using colorimetric assay. The amount of the protein loaded was checked with anti ß-tubulin antibody. The antibodies bound to the proteins were made visible on X-ray film. The quantitative analysis of the amount of phosphorylated and non-phosphorylated Cdc2 were carried out with the usage of proper analysing programmes on the X-ray filmes scanned.

RESULTS

<u>Isolation of *S. pombe* mutants with changed chromium sensitivity</u>

Chromium-sensitivity and chromium-tolerance was studied by using chromium-sensitive or chromium-tolerant mutants bearing single mutations, stabile genetic background and selective markers. These mutants were created by tetrad analysis.

Crosses introducing the *ura4-D18* marker to the strains:

The *chr2-04T*-t, *chr-09T*-t, *chr1-14T*-t, *chr-23S*-t and the *chr-33S* chromium tolerant (T) and sensitive (S) mutants derived from the *9chr*$^{+}$ (*leu1-32 h*$^{-}$) parental strain were crossed with the *89chr*$^{+}$ *(ura4-D18 h*$^{+}$*)* strain. We were looking for Cr(VI) sensitivive or tolerant strains with one gene affected and from these recombinants we wished to choose the ones carrying the uracil auxotrophic marker *ura4-D18* needed for further transformation experiments.

Cross nr. I (*chr2-04T x 89chr*$^{+}$)

We obtained the 2T: 2S segregation rate from the examined 25 full tetrads as a result of the tetrad analysis which suggests single gene mutation. Four of the chromium tolerant recombinants (MICcr(VI): 275 µM) were carrying the *leu1-32* or *ura4-D18* auxotrophic markers. The mutant *chr2-046T* was used for further experiments. Random spore analysis of the *chr2-04T* and *chr1-66T* strains showed a potential non-allelic mutations suggested that the mutation affected two different genes (Czakó et al., 2004). Only 44 of the examined 104 spores were Cr(VI) tolerant (MICcr(VI): 275 µM). The method used for selecting the transformants (Koósz et al., 2008) is depending on their genotype and therefore was not useful for the strain *chr2-046T* but the mutant *chr1-663T*, this is why *chr2-046T* was not used for future transformation.

Cross nr. II (*chr1-14T x 89chr*$^{+}$)

28 from the totally obtained 29 were full tetrads (Czakó et al., 2004). The outcome of testing for chromium sensitivity demonstrated 95% % +/- 5 % 2T:2S segregation. During the experiments the number of the cells were set up properly but there might have been some slight differences in the cell phases of the studied cultures which could result the +/- 5 % difference obtained. 400 spores during 8 crossing procedures were pulled. 266 out of the 400 spores were viable, that means 66.5% viability. 18 uracil auxotrophic spore clones were selected from these experiments.

One of the 18 Cr(VI) tolerant recombinants was condemned to random spore analysys. Crossing with the strain *chr1-66T*, 84 spore clones were obtained and 73 of them were tolerant to Cr(VI). This result may support the idea that both of the mutants crossed were bearing mutation in the same single gene.

For future experiments chromium-tolerant spores clones were chosen with single uracil or leucin or carrying both the uracil and leucin auxotrophic markers. These clones *92/7b*, *92/7d*, *92/8a*, *92/8d*, *96/3d*, *97/14c*, *97/14d*, *97/18a*, *97/18c*, *98/1a*, *98/1b*, *98/3a*, *98/5c*, *98/7a*, *98/9a*, *98/9c*, *98/10a*, *98/13d* all of them are derived from crossing the strains *89chr*$^{+}$ and *chr1-14 T*. Since the tetrad analysis procedures were carried out for obtaining chromium-sensitive or tolerant mutants determined by single mutations, bearing a stabile genetic background clones *92/7b*, *92/7d* and *97/14c*, *97/14d* from crossing strains *89chr*$^{+}$ and *chr1-14 T* have been chosen for further investigation.

Cross nr. III (*chr23S x 89chr*$^{+}$)

Cross nr. IV (*chr33S x 89chr*$^{+}$)

From these two crosses no asci have been obtained and so no tetrad analysis was accomplished.

Cross nr. V (*chr1-09T x 89chr*$^{+}$)

From four experiments we have got 56 full tetrads, 176 out of the 224 spore clones were viable which means 78,57 %- viability. 6 out of the 31 full tetrads showed 2:2 segregation.

Although we have found 2:2 segregation in the auxotrophic markers in some cases, after analyzing the results of the tests for chromium tolerance we have found no spore clones to fulfill the research criterias. Moreover most of the strains were not feasible under the normal growth conditions and so no spore clones were used for future investigations from the crosses of the strain *89chr*$^{+}$ and *chr-09T*.

Cross nr. VI (*chr1-662T x 21chr*$^{+}$) (The 2nd cross for the mutant's *chr1-662T* transfromation ability)

Induced mutagenesis of the strain 6chr^{+} (*lys1-131 h*$^{-}$, $MIC_{cr(VI)}$: 250 µM) the *chr1-66T* (*lys1-131 h*$^{-}$, $MIC_{cr(VI)}$: 275 µM) mutant was obtained. This strain carrying one-gene mutation was crossed with *90chr*$^{+}$ (*ura4-D18 h*$^{+}$, $MIC_{cr(VI)}$: 250 µM). *Chr1-661T* and *chr1-662T* are the two spore clones originated from this cross bearing (*662Tlys1-131 ura4-D18 h*$^{-}$, $MIC_{cr(VI)}$: 275 µM). Since the mutant *chr1-662T* showed no capability for transformation with pUR18N, that's why the 'cleanup' of the genetic background needed. For these experiments the strain 21chr^{+} was used as crossing mate (Czakó et al., 2004, Koósz et al., 2008).

During the investigation 100 spore clones were obtained, 88 of them vere viable, which means 88 % viability and 15 full tetrads were received. The two spore clones *12/9c* and *12/9a* (with the later publication code *chr1-663T lys1-131 ura4-D18 h*$^{-}$) showed chromium tolerance and the auxotrophic markers needed with 2:2 segregation.

In order to make sure, the strains we have created are determined by only a single mutation, with a stabile genetic background that is why the mutants were comdemned to a 'cleaning' cross. The spore clone *12/9a* was crossed with the wild type parental strain.

Cross nr. VI./a (*12/9a x 21chr*$^{+}$)

165 from the 180 aquired spores werre able to proliferate, so the 91.66 % viability as we expected was higher than int he cross nr. V. 29 out of the 32 full tetrads showed 2:2 segregation respecting the parental properties. After the chromium tolerance tests chromium tolerant strains with uracil auxotrophy were chosen, 2 spore clones from each 5 full tetrads, the clones *21/6c, 21/6d, 21/11a, 21/11c, 21/24b, 21/24c, 21/25a, 21/25d, 21/31b, 21/31c.*

The chromium tolerant mutant *chr1-663T* obtained from the strain *chr1-662T* was transformed with an expression vector encoding GR and *pgr1+*. The chromium sensitivity and the GR activity of the transformants point on the importance of GR-NADPH system (Koósz et al., 2008).

Cross nr. VII (*chr1-046T x 21chr*$^+$)

As a result of the experimental procedures 172 spores were received, 169 of them were viable which means 98.25 % viability. 11 from the 40 full tetrads showed 2leu$^-$:2ura$^-$ leu$^-$ or 2p$^+$:2ura$^-$ leu$^-$ segregation. Since we were looking for those chromium tolerant mutants carrying both leu and ura selective markers those 8 full tetrads were futher examined bearing prototrophic or leu and ura auxotropic.

Considering the results of chromium tolerance tests the *31/15a* and *31/15b* were the only chromium tolerant spore clones showing both leu and ura auxotrophy.

After all only 1 among the 40 full tetrads was bearing the 2:2 segregation of the required charateristics, so we supposed the obtained spore clones were having more than one mutant genes. In order to receive mutants carrying single gene mutation the *31/15a* followed cross.

Cross nr. VII./a (*31/15a x 21chr*$^+$)

Among the 196 spores 174 were viable, which appears 88.77% viability. There were only 2 spore clones useful for futher transformation the *41/9c* and *41/9d*. These chromium tolerant mutants were bearing the auxotrophic markers we were looking for, they were chromium tolerant although the MIC was 25 µM lower than the chromium mutant parental strain. Since the viability became lower and the maintance of the clones became harder during the crosses we assumed more than one gene mutations. Crosses from crosses these characteristics should have actually been improved. So these mutants were not used for further investigation.

From the crosses above the strain *chr1-663T (lys1-131 ura4-D18 h*$^-$, $MIC_{cr(VI)}$ 250 µM*)* was derived and used efficiently for later transformation experiments (Koósz et al., 2008).

<u>Experiments on Vpr protein and oxidative stress</u>

Cell morphologies and cell division of Vpr-expressing *S. pombe* cultures has been examined under different H_2O_2 stress conditions.

As described earlier we have also shown that VprNL4-3 protein expression resulted in cell elongation nondividing cells (the Cdc2 phenotype) and cell death.

1 µM thiamine content of the medium increased the cell proliferation of Vpr-expressing cells but it did not cause any alteration in cell morphology.

1 µM thiamine and 0.15 mM H_2O_2 addition exerted synergistic effect on the cell proliferation and the survival rate increased in the Vpr-expressing culture.

One-cell cycle treatment of cells with 0.15 mM H_2O_2 resulted in 15 % elongated cells in the Vpr-repressing cultures also shown with flow cytometric analysis.

Under adaptive and acute stress conditions no elongated cells have been observed and the average cell length decreased, respecting the controll cultures. The same H_2O_2 treatment did cause any paralell changes on Vpr-expressing *S. pombe*.

Our experimental data suggest that H_2O_2 treatment means a timely compensation against the pathological effects of Vpr expression while the cell cycle characteristics and the cell cycle arrest in G_2 phase are permanently the same in the Vpr-expressing *S. pombe* cultures.

We have shown that the Vpr repressing cultures shown 20 % cell death as adaptive response and 80 % cell death as acute response in 2 hours. The survival of Vpr-expressing cells increased by 15.8 % under adaptive stress conditions and increased by 80.3 % under acute stress conditions in a 2 hours long experiment.

<u>Changes in *S. pombe* cell cycle characteristics induced by MP</u>

In order to find out what kind of intracellular target molecules and pathways are affected by the Movement Protein we have used genetic screening. I have transformed all the mutants by electroporation. The transformants have been tested with a couple of different methods.

I’ve found that under MP expressing conditions the *S. pombe* cultures show retarded growth both in liquid and solid medium. The MP encoded by the ORF4 of the BYDV P4 arrested cell division.

The experimental datas show that the wild type fission yeast cells are about 10,4 ± 0,2 μm long, the MP-expressing ones are 14,8 ± 0,4 μm. We have shown that the wild type cells show their maximum length at 14 μm, and under MP expressing conditions 27 μm. We have found that in the case of the wild type *S. pombe* cells the septation index is about 8 to 15 % and if the MP is expressed the septation increases up to 34 %.

The presence of septated cells under the BYDV MP expression suggest that cell cycle arrest happens might not or not only at the G_2 to M transition but may in M phase.

The evidences suggest that the cell cycle mutant genes (*rad 3, check 1, cds 1, check1 cds 1*) show no change on MP's effect which means that none of these checkpoint molecules are targeted by the MP.

One of the PP2A enzyme mutants, the *Δpab1* was only partially supress the MP. These cells showed no morphological changes but growth arrest if the MP was expressed in these cultures.

The *Δppe1* („pp2A like enzyme") mutant showed full supression on Movement Protein. These cells showed normal phenotype in cell length and healthy growth, colony formation. Those results mean that pp2a like enzyme is a target of the MP protein, the PP2a is only partially involved. Probably this happens because of the functional overlapping between the 2 enzymes.

The *cdc2c 1w* and *cdc2c 3w* strains lacking 2 different point mutations of Cdc2. The *cdc2 1w* showed full supression on MP's effect, and this mutation is the one sensitive for Wee1 phosphorilation while the *cdc2c 3w* is not.

We showed the phosphorilation status of the cell cycle checkpoint key molecule Cdc2 with western blot analysis. When the Movement Protein was expressed there was practically most of Cdc2 is phosphorilated which means cell cycle arrest.

Other results suggest that Wee1 is one of the target molecules because this strain shows no changing in septation under MP expressing conditions and this is why we assume that Cdc25 is also involved in the pathway Mp acts through.

The experiments on GFP-tagged MP we have proved that in the non-supressing mutants the MP is always associated to their nucleus. Only the Pp2a like enzyme mutant shows no GFP-Mp aggregation close to the nucleus what was a sort of a morphological evidence of the full MP supression.

SUMMARY

1. Working on hundreds of tetrad analysis in a crossing project chromium-tolerant strains (spore clones) were obtained bearing single gene mutations with stabile genetic background and the auxotrophic marker (*ura4-D18*) was also introduced. The chromium tolerant mutant *chr1-663T* (MIC 250 μM) were chosen for transformation. Furhter experiments with this mutant transformed with an expression vector bearing the *pgr1+* gene encoding glutathione reductase seems to support the idea that GR-NADPH system plays an essential role in chromium tolerant characteristics of *S. pombe* (Koósz et al., 2008).

2. The viral proteins has been already known as oxidative stress causing agents. However, the interaction between HIV-1 Vpr and oxidative stress has been mentioned only a few times in the literature. Our results indicate that certain doses of oxidative stress do not enhance the pathology of Vpr but results in protection against cell death caused by Vpr expression. Certain levels of H_2O_2 treatment in Vpr-expressing cultures was able to increase the cell survival during a special period of time. Vpr-expressing cells responded to the acute oxidative stress caused by 25 mM H_2O_2 with 80.3 %- increasing cell survival in the short term about one cell cycle. And the adaptive H_2O_2 treatment which means a 1 hour 0.15 mM H_2O_2 pretreatment followed by 25 mM H_2O_2 as final concentration also seems to increase cell viability by 15.8 %. The activated cellular mechanisms did not act against cell cycle arrest in G_2 phase but might operate during this blocked cell division. These observations possibly mean that the cell response induced by the Vpr expression confers protection against H_2O_2 stress and the cellular mechanisms activated by H_2O_2 are able to inhibit the cell death caused by Vpr.

3. Before our experiments were carried out no experimental details were published about MYDV MP. Fission yeast mutants were used in order to find out how MP affects the cell proliferation. The wild type *S. pombe* strain (*SP223*) and the cell cycle mutants (*rad 3-139, chk 1, cds 1, chk1cds 1, ppa2*) showed elongated cells with multiple septas and unequal segregation of chromosomes. I was basically looking for those MP suppressing mutations which show no morhological changes on *S. pombe* cells and no changes on cell cycle characteristics.

4. Using western blot analysis I showed that under MP expressing conditions most of the cell cycle key molecule the Cdc2 is phosphorylated. In this case the the cells stop dividing. The *cdc2 1w* mutant showed the same properties whether the MP was expressed in this strain or not. This mutation carries a special poin mutation which blocks the phosphorilation most of the Cdc2 molecules. This data also suggest that MP acts on the mostly phophorilated Cdc2 protein.

5. During the research I've found that certain strains (*cdc2-1w, ppe1, wee1-50Δmik1*) show no changes in cell cultures under MP-expressing conditions. These mutations ensure one protein loss in function. In these cases MP could not take effect on the mutated proteins. These are the molecules responsible for cell cycle arrest suggesting the possible elements of the pathway for example Cdc25, Cdc2 or Wee1 MP acts through. Ppe1 the 'Pp2A like enzyme' which plays role for example the equal segregation of chromosomed during the cell division was also one of the target molecles of MP.

6. If MP is expressed causes multiple sepated cells, unequal segregation of chromosomes as mitotic abnormalities in *S. pombe* cultures. These kind of abnormal cells are unable to perform further division. As we prooved, the MP expession itself is able to induce the damaging effects of BYDV. This is why we can say that the experimental data presented is to support the idea that the MP encoded by the BYDV is a viral determinant and MP can be itself responsible for the retarded growth and agricultural damages.

PRESENTATION OF NOVEL FINDINGS

1. *Chr2-04T* and *chr1-663T* chromium-tolerant mutants were determined by single mutations, bearing a stabile genetic background and carrying useful selective markers for further experiments in order to analyze the chromium tolerant and –sensitive characteristics.
2. Low level (15 mM) of H_2O_2 decreased the number of elongated cells from 98 % to 43 % in Vpr-expressing cultures. During the first cell cycle after the H_2O_2 treatment the treated cells under Vpr expression were partially protected against the killing effect of Vpr protein. The 0.15 mM H_2O_2 content in medium induced stress-defence mechanism against and so increased the survival of Vpr-expressing *S. pombe* cells.
3. We have shown that Vpr-expressing cells respond with an increasing cell survival in the short term about one cell cycle to acute stress conditions 25 mM H_2O_2.
4. During the experiments our findings suggest that the adaptive H_2O_2 treatment was able to increase the survival rate of Vpr-expressing *S. pombe* cells although the acute H_2O_2 treatment was much more effective. The intracellular mechanisms acting against H_2O_2 stress did not have any effect on the G_2 cell cycle block. These results support the explanation that the intracellular pathways activated by oxidative stress are also involved in inhibitory cellular processes against Vpr. Our results suggest that certain doses of oxidative stress do not enhance the pathology of Vpr but result in protection against cell death caused by Vpr expression.
5. We have developed a fission yeast model system containing 39 *S. pombe* strains to study potential effects of BYDV MP on basic cellular functions.
6. We showed that under MP-expressing conditions the cultures contain elongated cells which are arrested at their division. The MP causes mitotic abnormalities such as multiple septation, unequal segregation of chromosomes, and the aneuploidy of the daughter cells. Our data suggest that Mp does not only affects the normal cell cycle but also responsible for unequal segregation of chromosomes. And so we prooved that the MP viral determinant encoded by the BYDV demostrates most of the syptoms of the whole viral desease even if it is expressed in fission yeast cells.
7. Target molecules of MP have been identified such as Cdc2, Cdc25 and Wee1. These are the key molecules of the classical G_2/M checkpoint pathway. So MP acts through partially unknown mechanisms, although some of the elements influenced are the same as the proteins responsible for the G_2/M cell cycle.
8. The PP2A-like enzyme which possibly ensures control of the mitosis and so is responsible for normal cell cycle was also identified as a target molecule of MP.

LIST OF PUBLICATIONS

Publications related to the thesis

1. Czakó-Vér, K., Koósz, Zs., **Antal, J**., Rácz, T., Sipiczki, M. and Pesti, M. (2004) Characterization of chromate-sensitive and -tolerant mutants of *Schizosaccharomyces pombe*. Folia Microbiologica 49, 31-36. (IF: 0.979)

2. **Antal, J**., Zongliang, X., Benko, Z., Zhiqiang, D., Shi, F., Liu, K., Pesti, M., Wang, D. and Zhao, R., Y. (2011) BYDV MP is a viral determinant for retarded plant growth, cell cycle G_2/M arrest and mitotic abnormality (in manuscript)

3. **Antal, J**., Pesti, M. (2005) The dose-dependent H_2O_2 stress response promotes increased survival for *Schizosaccharomyces pombe* cells expressing HIV-1 Vpr Folia Microbiologica . (IF: 0.979)

4. Koósz, Zs., Gazdag, Z., Miklós, I., Benkő, Z., Belágyi, J**., Antal, J.**, Melegh, B., Pesti M. (2008*) Effects of Decreased Specific Glutathione Reductase Activity in a Chromate-Tolerant Mutant of Schizosaccharomyces pombe*. Folia Microbiologica 53, 308-314. (IF: 0.979)

Conference abstracts related to the thesis

1. Czakó-Vér, K., Koósz, Zs., **Antal, J.,** Grama, L. and Pesti, M. (2003): Investigation of chromium-tolerant mutants of *Schizosaccharomyces pombe* by cytometry and tetrad analysis. Acta Microbiologica Et Immunologica Hungarica, 48: 161.

2. **Antal, J.,** Pesti, M. (2005) *Conspiracy theory on MAPK pathway elements as inhibitors of HIV-1 Vpr protein*. Acta Microbiologica et Immunologica Hungarica 52, pp. 31st CEFORM Keszthely, Hungary

3. **Antal, J.,** Xia, Z., Benkő, Zs., Du, Z., Shi, f., Liu, K., Pesti, M., Wang, D., Zhao, R., Y. (2005) *movement protein of plant pathogenic BYDV causes mitotic abnormalities and cell cycle arrest in fission yeast.* Acta Microbiologica et Immunologica Hungarica 52, pp. 31st CEFORM Keszthely, Hungary

Conference abstracts not related to the thesis

1. Czakó-Vér, K., Koósz, Zs., **Antal, J.,** Grama, L. and Pesti, M**.** (2001) *Króm-toleráns Schizosaccharomyces pombe mutánsok jellemzése áramlási citometriával és tetrád analízissel.* Az 50 éves Magyar Mikrobiológiai Társaság 2001. évi Jubileumi Naggyűlése Balatonfüred, Hungary, pp. 26.

2. **Antal, J.,** Xia, Z., Benko, Z., Fenyvesvolgyi, Cs., Du, Z., Shi, Z., Liu, K., Yu, M. (2004) *BYDV MP is a Viral Determinant for Retarded Plant Growth as Results of Cell Cycle G_2 Arrest and Mitotic Abnormality.* 3rd International Fission Yeast Meeting, San Diego, U.S.A.

3. **Antal, J.,** Pesti, M. (2005) *Conspiracy theory on MAPK pathway elements as inhibitors of HIV-1 Vpr protein* 1st Central European Forum for Microbiology (CEFORM), Keszthely, Hungary