were reflected in expression level of *Nos2, Ptgs2*, the most important genes responsible for murine MSC-mediated immunosuppression. The strongest inhibitor MSCs expressed the most and the least inhibitor clones expressed the lowest level of these factors at mRNA level. Normally, MSCs exert immunosuppression at the site of inflammation, therefore the immunmodulation of MSC clones were tested in inflammation-mimicking milieu, treating MSC clones with pro-inflammatory cytokines, IFN-γ and TNF-α. Treatment of the cells with these cytokines resulted in upregulation of *Nos2* and *Ptgs2* gene expression in each MSC clone, and as a consequence, their inhibitory effect on T cell proliferation elevated. To find out whether MSC clones can exert immunmodulation *in vivo,* the effect of the most and the least immunosuppressive MSC2 and MSC6 clones, respectively, were tested in ovalbumin-induced delayed-type hypersensitivity response in mice. Intraperitoneal administration of MSC2 cells simultaneously with ovalbumin immunization significantly reduced, whereas MSC6 didn't change the ovalbumin-induced increase of footpad thickness, unless MSC6 cells were pretreated with IFN-γ and TNF-α prior to injection, in that case MSC6 also decreased footpad thickness increment vigorously.

Based on our results, we suggest that murine BMMSC population is homogenous in differentiation and angiogenesis support while heterogeneous in immunosuppression. Dissimilarity in the immunosuppressive function likely depends on the activation state of single MSC cells, since placing the cells into an inflammatory milieu, the immunomodulatory effect of different MSC clones becomes similar.

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Genetic analysis of *Saccharomyces cerevisiae RAD5* gene

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The sequence of DNA contains important information for the life of cells. Any damage of DNA leads to inaccurate function of the cell, or occasionally to its death. Therefore, proteins of DNA repair have a critical role in preserving the initial state of DNA.

At DNA damage, the replication polymerase stalls and the complex of Rad6 and Rad18 proteins ubiquitylates the Proliferating Cell Nuclear Antigen (PCNA), the processivity factor of replication polymerases, at its lysine 164 residue. Subsequently, the monoubiquitylated PCNA is polyubiquitylated by the protein complex of Rad5, Mms2 and Ubc13.

The Rad5 has three domains: RING, Helicase/ATPase and Hiran domain. While the RING domain has E3 ubiqutine ligase activity and the Helicase/ATPase domain has a replication fork reversal activity facilitating the formation of a chicken-foot DNA structure, the function of the Hiran domain is unknown despite of its predicted DNA binding capability.

To explore the particular functions of these Rad5 domains we tested the sensitivity of *ring* (CC914,917AA), *atpase* (DE681,682AA) and *ring-atpase* double point mutant strains with different mutagenic agents such as UV-light, methyl methanesulphonate and nitrogen-mustard.

We have found that the single mutant strains (*ring*, *atpase*) were sensitive to all tested mutagens and the double mutant strain (*ring-atpase*) had a higher sensitivity than the single mutants. We concluded that the ubiquitin ligase and the ATPase activities of Rad5 are not epistatic. This implies that these two activities have independent functions, but it is not exclude the existence of a common function. We also intended to investigate the relationship of these two activities of Rad5 with *RAD18* and *RAD51* DNA repair pathways. Although in our previous results epistatic relationship was not manifested among *RAD5*, *RAD18* and *RAD51* genes with none of the tested mutagens, one could not exclude the possibility that either the ligase or the ATPase activity of Rad5 could interact with one of these pathways. To explore this possibility the epistatic relationship of *ring* and *atpase* mutants was analyzed on *rad18Δ* and on *rad51Δ* background. On these deletion backgrounds point mutants represented the similar sensitivity like on wild type background. These results suggest that domains of Rad5 could function in the same DNA repair pathway with both of the proteins Rad18 and Rad51. Or probably none of the domains function with these two proteins, only they function with one or more other proteins out of Rad18 and Rad51. This hypothesis was confirmed by the higher sensitivity of *rad5Δ/rad18Δ/rad51Δ* triple mutant strain than *rad5Δ/rad18Δ*, *rad18Δ/rad51Δ* and *rad18Δ/rad51Δ* double mutants. Although this sensitivity could be caused by other functions of the Rad5 (e.g. function of Hiran domain). To prove this theory we intended to test the sensitivity of the point mutants on *rad18Δ/rad51Δ* background.

To explore the role of the Hiran domain, we generated mutations in its conserved regions. Five from the twelve mutant strains showed sensitivity to DNA damaging agents (nitrogen-mustard and hydroxyurea). Two mutant strains from the five showed the same growth curve like wild type on mutagenic treatment if over-expression of proteins were induced. It means the low expression level of these two mutant proteins caused their sensitivity in our previous experiments. The other three were overexpressed, purified and tested *in vitro* in biochemical assays. The LI265,266RR mutant protein exhibited wild type activity while the GA177,178RR and the G183R mutants showed no activity neither in helicase nor in ubiquitin ligase assays. We concluded that the GA177,178 and the G183 parts of Hiran domain are likely to have a basic role in both of the two functions of Rad5. Nevertheless it is possible that these mutations modify the whole structure of the protein and it loses all of its activities. To answer this question more structural studies are needed with both wild type and mutant proteins.

We concluded that the role of Rad5 out of the *RAD18* pathway is none or just partially related to *RAD51*. In addition, the ubiquitin ligase and the ATPase/helicase activities of Rad5 have independent function from each other, and these functions are not exclusively func-

tion with Rad18 or Rad51. Probably these independent functions act with one or more other proteins out of Rad18 and Rad51. Thus we expect that Rad5 has a more complex protein interaction network than it was previously known.

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Heterologous protein expression and *in vitro* analysis of *Drosophila melanogaster* proteins involved in telomere maintenance

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Our laboratory is particularly interested in the maintenance of genome integrity and telomere structure in *Drosophila melanogaster*. In eukaryotic cells, telomere prevents the chromosome ends from being detected as DNA double strand breaks and protects the coding regions from degradation. Telomere "capping" by the multi-subunit complex Shelterin, expressed in higher eukaryotes, averts the triggering of the DNA damage signaling pathways. In *Drosophila* this capping process is performed by a putative complex called Terminin, which is believed to have HOAP, HipHop, Ver and DTL (or Moi) proteins as subunits and it binds to the DNA in a sequence-independent manner. HP1 is generally regarded as the fifth subunit of the putative complex, thought it is not a strictly terminin-specific protein. HP1 is evolutionary highly conserved and plays a role also at non-telomeric regions while Terminin proteins manifest an accelerated rate of evolution and localize only at chromosome ends. Deletion of any of these genes results in telomere fusions. The physical interactions among Terminin proteins have been demonstrated *in vitro*. All these data support the existence of the Terminin complex.

The aim of my research was to study in details the putative Terminin complex and its suggested components by bioinformatics and molecular biological methods.

In silico analysis on the proportion of synonym and non-synonym codon substitutions confirmed that the full length HOAP, HipHop, DTL and Ver molecules have accelerated evolution compared to HP1. Interestingly, specific protein domains showed different rates of evolution and some of the hyper-variable domains have a role in protein-protein interactions.

The cDNA of each protein was cloned and expressed both in bacterial and in baculoviral expression systems. Our results indicated that DTL and Ver proteins form inclusion bodies in bacteria. Co-expression with at least two interacting partners resulted in soluble DTL and Ver proteins. A polycistronic construct containing all the five cDNA was engineered, and the purification of the complex is in progress. Early data suggest the formation of several sub-complexes rather than the assembly of a holo-complex in bacteria. The DTL protein produced in baculovirus system was applied in far-western experiments and although we were unable to detect interactions with Terminin proteins by this method, an interaction with a nuclear protein has been revealed.

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Biological control of plant pathogenic fungi by use of a *Bacillus amyloliquefaciens* strain

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The damage in agricultural production and storage by phytopathogenic fungi is a serious problem in agriculture. Biological control is an alternative method against phytopathogenic microbes. An organism, which can interfere with pests or pathogen species, is referred to as a biological control agent. A biological control agent can compete for niche and nutrients in the rhizosphere, inhibit the growth of plant pathogens by the production of antibiotics and extracellular lytic enzymes and act indirectly, promoting plant growth and triggering defensive systems of plants against pathogens. It is beneficial if antagonistic strains used for protection against pathogenic microorganisms are resistant to metals present in the soil. The antagonistic microorganisms have to grow and reproduce as well as produce antibiotics and extracellular enzymes in the presence of different metals. The use of biological control may not always be sufficient against pathogenic species. In this case, we need to use combined methods such as use biological control agents with pesticides and appropriate cultivation methods. The biocontrol agent needs to tolerate against the pesticides used in combined treatments.

The *Bacillus* genus contains various species with potential biocontrol capabilities. *Bacillus amyloliquefaciens* SZMC 22206 has been isolated and studied as a potential biocontrol agent in our laboratory. Our aims were to investigate the effect of metals and pesticides on the growth, the extracellular chymotrypsin-like enzymes and the antibiotics produced by *B. amyloliquefaciens* SZMC 22206 strain and to