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We established stable cell lines, which expressed the ΔCR-PrP mutant or wt $PrP^C$ with the reporter gene GFP. Subsequently, in order to achieve a high overexpression of wt $PrP^C$ or Sho in the ΔCR-PrP expressing cells lentiviral transduction is used. For assessing the drug hypersensitivity caused by the expression of ΔCR-PrP, the cell viability with or without Zeocin treatment is measured using an MTT assay.

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## A comprehensive view of the determinants of molecular evolution in yeast

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Why do genes evolve at different rates? It is a well-known phenomenon that amino acid sites of protein sequences undergo substitutions during the course of evolution, and the rate of this change varies widely across genes. In the past years the major determinants underlying protein sequence evolution have been largely uncovered. However, molecular evolution is not restricted to amino acid substitutions, but rather encompasses various aspects of molecular changes from the deletion and duplication of whole genes to change in expression levels and subcellular localizations. Importantly, while the rate of sequence and expression divergence has been thoroughly studied, gene duplicability and the propensity for gene loss remain poorly understood, and it remains completely unexplored what determines the propensity for change in subcellular localization.

Here we aim to systematically explore and compare the genomic and functional genomic properties determining i) sequence divergence, ii) gene expression divergence, iii) propensity for gene loss, iv) gene duplicability and v) propensity for change in subcellular localization of proteins.

We compiled a dataset of various evolutionary variables (*i.e.* evolutionary rates of the above-listed molecular traits) using available information on sequence, expression, gene annotation and protein localization from *Saccharomyces cerevisiae* and its homologous genes in related species ranging from *S. paradoxus* to *S. pombe*. We also compiled high-coverage functional genomic data on various genomic and functional properties of genes/proteins in *S. cerevisiae* (e.g. information on protein abundance, protein network connectivity, fitness contribution, genetic interaction connectivity, etc.). In addition to classical statistic tools, we employed a data mining regression tool (random forest) to predict evolutionary rates based on these gene properties. This enables us to compare the predictability and the main determinants of different aspects of molecular evolution.

First, we asked whether the different molecular traits of a gene evolve in a correlated fashion. We found that the different evolutionary rates show only very weak correlations with each other, suggesting that different gene properties diverge rather independently throughout evolution. Next, we examined the predictability of molecular evolution. Corroborating earlier reports, we found that sequence divergence is well-predictable, with 54% of variation in divergence rate explained. The rate of expression divergence and gene loss can also be predicted using genomic features (28% and 27%). Duplicability, however, showed little predictability. Furthermore, in contrast to other evolutionary traits, the rate of duplication is only marginally conserved when calculated on different branches of the phylogenetic tree. Taken together, these findings indicate that gene duplication is not driven by strong universal evolutionary forces. We found that the rate of evolutionary divergence in protein localization is also predictable and revealed novel factors determining the conservation of protein subcellular localization. For example, we found that highly expressed genes show especially strong conservation in localization.

In our study, we have systematically examined the driving forces behind evolutionary change of different gene-level molecular traits using data-mining methods and recent functional genomic datasets. While some evolutionary variables are highly predictable, we report that the diversity of duplicability across genes is lineage-specific and no strong universal determinant of duplicability exists. We also discovered a number of novel determinants of protein localization conservation.

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## Analysis of blood cell lineages in *Drosophila melanogaster*

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Insects are armed with a powerful innate immune response, which provides an effective barrier against invaders and tumors. The phylogenetically conserved immune functions, such as the phagocytosis of microbes and the encapsulation of large foreign particles are carried

out by specialized immune cells, the hemocytes.

The development of the hemocyte lineages of *Drosophila melanogaster* is the result of a strictly regulated succession of intracellular and intercellular events. Studies on the *Drosophila* immune system have provided most of our knowledge on hematopoiesis and blood cell lineages in insects, and have shed light on some of the key features of blood cell differentiation in the animal kingdom.

The differentiation of hemocytes begins in the early embryonic stages. Two distinct mesodermal segments give rise to two independent embryonic hemocyte lineages: the procephalic mesoderm differentiates into embryonic macrophages and crystal cells, while the cardiogenic mesoderm forms the embryonic lymph gland. In the larval stages, hemocytes occupy three hematopoietic compartments. The lymph gland is a compact hematopoietic tissue consisting of paired lobes along the anterior end of the dorsal vessel. The sessile hematopoietic tissue localizes to the inner wall of the body cavity, and forms a banded pattern along the length of the larva. Both of these hematopoietic tissues contain differentiated effector cells, as well as precursor hemocytes. The third compartment, the circulation, comprises two effector hemocyte types: the plasmatocytes and the crystal cells. The plasmatocytes are phagocytic cells which engulf microbes and produce antimicrobial peptides, while crystal cells contain enzymes neccessary for the melanization cascade. Attack by the parasitoid wasp *Leptopilina boulardi* results in the appearance of a third effector cell class in the circulation, the lamellocytes, which form multilayered capsules around large foreign particles. Although the process of blood cell differentiation has been studied extensively, the origin of the hematopoietic compartments and effector hemocytes are still not well recognized.

Our aim was to track the distinct hemocyte lineages from the embryo to the adult and in the course of cell mediated immune response in *Drosophila*. In order to achieve this, we performed *in vivo* cell lineage tracing in combination with the use of molecular markers.

Our results show that the two embryonic hemocyte lineages form discrete larval hemocyte compartments, all contributing to the emergence of the effector cell pool upon immune induction. When we followed the fate of the effector cell types, it also became evident that plasmatocytes display a peculiar ability to transform into lamellocytes, therefore highlighting the plasticity of the effector hemocytes and the *Drosophila* immune system in general.

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## Studying the function of the secretory pathway during germ cell formation especially of the Golgi associated retrograde protein (GARP) complex

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In many cases the malfunction of the sperm is the underlying factor in human fertility problems. However it is a significant issue, our knowledge is rather limited on the mechanism of the molecular structure and the development of the sperm.

It is well known that genes responsible for the phenotype and function of the sperm are evolutionarily conserved, including human, mouse and *Drosophila*. In the testis of *Drosophila* the whole process of spermatogenesis can be studied as from stem cell through every step to the mature sperm. During the last few years many membrane traffic mutants were identified with male sterile phenotype, what suggests that proteins involved in membrane trafficking has an important role to play in germ cell formation.

Vps54 (Scat) protein, a subunit of the Golgi associated retrograde protein (GARP) complex, is participating in the retrograde transport (transport from the extracellular space or organelles of the secretory and endosomal-lysosomal system towards the Golgi and the endoplasmatic reticulum).

Our research confirmed that Vps54 homozygous mutants show male sterile phenotype however females are fertile. Studying the mutant testis, abnormality could not be found at the early stages of spermatogenesis, however at later phases sperm cysts become unorganized and matured sperms were immobile.

Additionally we found that however viability of the homozygous Vps54 mutants were normal, the body-size of the mutants were conspicuously smaller according to the heterozygous ones.

Furthermore in flight tests mutants were found almost unable to fly and moreover, in larvae difficulties in peristaltic movement could be observed, what assumed defects in muscle or in neural system. Studying the muscle tissue of mutants although no structural aberration was found by fluorescent microscopy, loose myofibrils were visible with EM. For additional investigation of Vps54 mutant phenotype GFP, RFP and 6xMYC tagged Vps54 transgenic *Drosophila* lines were established to examine the localisation of the protein *in vivo* during spermatogenesis and myogenesis.

We do hope that our research contributes to gain a better insight into the function of Golgi associated retrograde protein transport during spermatogenesis. Also studies of GARP complex may open new perspectives as retrograde protein transport is scarcely investigated even so its importance is recognized.

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