Analysis of cell growth, division and size control mechanisms in fission yeast

Summary of the PhD thesis

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1 Introduction

During my PhD work I studied the fission yeast cells’ growth, division and size control mechanisms during their mitotic cycle. Studying growth is important for several reasons, for example, it is essential for doubling the cell mass which is important for the size homeostasis during consecutive generations. Moreover, it has a main role in some cells movement (e.g. leukocytes and amoebae) and in creating polarized structures (e.g. axons in neurons). Since the basic strategies of the growth are evolutionary conserved, it is handful to study model organisms which reproduce rapidly and are easy to study. The fission yeast, *Schizosaccharomyces pombe*, is a favourable model organism of cell growth studies, since its cells grow exclusively at their tips, therefore cell volume is proportional to cell length, which is an easily monitorable parameter. There is still a debate regarding of which model is the best to describe the growth in fission yeast cells. To date no broad study was performed analysing individual cells’ growth patterns. Therefore the aim of my PhD work was to study the cell growth in eight fission yeast cultures (wild-type and several cell cycle mutants). The size homeostasis of the cells in a population is achieved via size control mechanisms, which ensure that only cells reaching a critical size can divide. I have studied cell growth and size control with time-lapse microscopic films. Moreover, I have analysed some proteins which have an essential role in cell growth and division by phylogenetic methods, to determine the evolutionary relation between them.

2 Background

Cell growth is intensively studied during the last few decades in several model organisms. Growth in different cells can be described by different functions, however there are also some general strategies.\(^1\),\(^2\),\(^3\)

Cell growth in fission yeast was first investigated my Murdoch Mitchison. He showed that there is no cell growth at the last ¼ of the cell cycle, which is referred as constant length phase.\(^4\) Growth is limited here as the cell cortex plays an important role during mitosis and cell division. It was shown that after division fission yeast cells grow only at the old end (OE), which had already been a growing end in the previous cycle. At around the ½ of the cycle the new end (NE; formed by the last division) also starts to grow, so the unipolar growth switches to bipolar, and this event is called the new end take off (NETO) (Figure 1). This causes a ~40% increase in the growth rate, as the cell grows faster bipolarly, however as the new end starts growing, the growth rate of the old end

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decreases. Therefore length growth in fission yeast can be best described by a bilinear function, which consists of two linear segments separated by a rate change point (RCP). There is still a debate whether the bilinear function is the best to describe length growth in fission yeast. Moreover, if the bilinear function was the best, then there is a question whether the growth rate increases abruptly causing a sharp rate change, or there is a continuous change in growth rate resulting in a smoother transition period. Therefore the aim of my work was to study the growth patterns of around 590 individual fission yeast cells to find the most adequate model to describe cell growth, and to test whether there is a universal strategy or not.

Figure 1. The fission yeast cell cycle and the cytoskeleton rearrangements during it.

The existence of a size control mechanism was proved in several organisms including unicellular and more complex higher organisms as well. Several strategies can ensure size homeostasis, and different organisms may have different size control mechanisms. The existence of size control in fission yeast was first proved by Fantes. He plotted both cycle time and length extension during the cycle versus birth length. Negative correlations were found in both cases, which indicated that there was a size compensation mechanism in the cycle. Later the position of the size control was determined with the help of the RCP in bilinear patterns (see above), which was found to be mid G2 in wild-type cells.

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Cell growth and division must occur strictly coordinated. To achieve this, several proteins have to work strictly coordinated like cell wall synthases and hydrolases. By bioinformatics methods, I have studied the proteins responsible for the synthesis and hydrolysis of α-glucan which is a main component of the fission yeast cell wall. Five α-glucan synthases are known in fission yeast, and one of them, the Ags1 protein has an essential role in cell wall synthesis during the mitotic cycle. As a result it localises always at the growth zones (it is present before NETO at the old end, after NETO at both ends, and from mitosis in the middle of the cell). The Mok11, Mok12, Mok13 and Mok14 α-glucan synthases play important roles during the sporulation cycle. Moreover α-glucan synthases are important in several human and plant pathogens, as the presence of α-glucan in the cell wall or in the capsule is in connection with the pathogenicity of several fungal species.

The last step of the fission yeast cell cycle is cell separation, which starts with the hydrolysis of the septum edging by the Agn1 α-glucanase (Figure 1). Another α-glucanase known is fission yeast is Agn2, which hydrolyses the spore wall during sporulation. The α-glucanases have several other functions in different species, for example some fungi express this protein against mycoparasites to kill them. Another species produce α-glucanase during starvation to mobilize α-glucan which can be used as carbon and energy source. Moreover some α-glucanases may also have pharmaceutical significance, as some pathogenic species lose their pathogenicity after α-glucan hydrolysis of their cell wall or capsule. There are some toothpastes and mouthwashes which contain α-glucanase, to reduce the dental plaque formation as one of the main components of the plaque is the α-glucan capsule produced by the Streptococcus mutans bacteria.

3 Methods

I have analysed cell length growth in fission yeast on time-lapse microscopic films taken by Murdoch Mitchison (University of Edinburgh, UK). During filming, the cells grew on a thin agar surface in the microscope and pictures were taken in every five minutes. Later the pictures were projected and cell length could be monitored from birth to

division. I have analysed about 590 individual fission yeast cells’ growth patterns from eight different cultures (A: wild-type haploid (972h), B: wild-type diploid, C: wee1Δ mutant, D: cdc2-3w simple mutant, E: cdc2-3w cdc25Δ double mutant, F: cdc2-3w cdc25Δ pyp3Δ triple mutant, G: cdc2-33 mutant steady-state, H: cdc2-33 mutant induction synchrony culture). After a numerical smoothing of the measured growth patterns, the borders of the growth period were determined. First the start of the growing was determined (RCP1), as in the first 5-10 minutes of the cycle some growth abnormalities can be observed, mainly caused by the turgor pressure in the cells. Then the start of the constant length phase (RCP3) was determined (Figure 2). I have fitted three different functions to the growing period (between RCP1 and RCP3) of every individual cell:

- linear: \( L_1(t) = \gamma t + \delta \)
- exponential: \( L_2(t) = \kappa \cdot e^{\mu t} \)
- and bilinear: \( L_3(t) = \eta \cdot \ln \left( \frac{e^{\alpha_1(t-\tau_{RCP2})}}{\eta} + e^{\alpha_2(t-\tau_{RCP2})} \right) + \varepsilon \)

As the fitted models have different parameter numbers, it is important to use model selection criteria which consider both the parameter number and the fitting of the model as well, because the most adequate model can be chosen only by these criteria. I have applied more criteria during my work, however Akaike Information Criterion (AIC) was found to be the most appropriate.

I have not only analysed the growth of the individual cells, but have also constructed a hypothetical average cell of every steady-state culture. For this purpose, I normalized the growth pattern of every cell both to its birth length and cycle time and then all these patterns were calculated to a unified scale with linear interpolation. These growth patterns were averaged and the averaged pattern was multiplied with the cultures average birth length and cycle time values. These average cell length growth patterns were analysed with the same methods as that of the individual cells.

**Figure 2. Taking time-lapse microscopic films and the determination of the growth period [4.]. Abbreviations used in the figure are listed in Chapter 4.2.**

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The growth pattern analysis may help us to understand the size control mechanisms operating during the cycle. I have performed regression analyses for several growth parameters to study the size control.

For the phylogenetic analysis of the α-glucan synthases and the α-glucanases first I searched for related sequences among Fungi with Blastp search in different databases using the fission yeast proteins’ amino acid sequence as query. Then I analysed the domain structure of the potential homologous sequences with the Pfam and NCBI CDD databases, to improve the reliability of the hits. Several multiple alignment methods (ClustalX, PRANK, MAFFT) were applied to achieve a more reliable result. Then more phylogenetic tree constructing methods (neighbour joining, maximum parsimony, maximum likelihood, Bayesian) were used to generate different trees from the full protein sequences and also from their domain sequences. As there is no broadly accepted method, neither for multiple alignment nor for tree constructing, one has to test different methods parallel. All these methods approximate the evolution in slightly different ways, so if we get similar results, we probably find the phylogenetic tree showing the real relationships. The evolution of proteins and genes may show several differences compared to the evolutionary relationships of the species, as for example gene duplication or gene loss events may modify the relations. As a consequence, not only the evolution of the species is important, but studying the evolution of proteins and genes has also high impact.

4 Results

4.1 Length growth patterns during the mitotic cycle of fission yeast

Studying the growth patterns in the eight analysed cultures revealed that the fission yeast cells’ growth cannot be described with one model, as it shows heterogeneity. However, most of the cells followed a bilinear pattern (50-82% in different cultures), but linear models were also frequent (6-45%), especially in cells larger than wild type. Exponentially growing cells were found very rarely (2-12%), and it might be probably caused by measurement uncertainties. About half of the bilinearly growing cells had a sharp rate change, but the other half rather had a smooth rate change, meaning that there was a transition period around the RCP with a continuously accelerating growth rate (Figure 3).

I have also analysed the average cells’ growth pattern constructed from individual data of every steady-state culture. The bilinear model was found to be the most adequate in all the seven cases, which confirmed that the growth of the fission yeast cells can be best described by a bilinear function. This may be in connection with the NETO event in some cultures (e.g. wild type), however in other cultures the gene dosage effect may be responsible for the rate change (e.g. wee1Δ mutant), since after S phase gene expression is generally doubled (Figure 3).
Figure 3. The distribution of the growth patterns in different fission yeast cultures and the growth pattern of the average cell of the cdc2-3w mutant [4., 5.].

4.2 Size control mechanisms in fission yeast

To study the size control operating during the cell cycle, I have analysed further the growth phase of the cells. I have performed regression analyses to determine whether there is any correlation between the cell length at the start of the growth phase ($L_{RCP1}$) and the duration of the growth phase ($T$) and length extension ($Ext$). I successfully proved the existence of an operating size control in every culture, as negative correlations were found between Ext and $L_{RCP1}$, and also between $T$ and $L_{RCP1}$. To determine the position of this operating size control, the growth phase was divided into two at RCP2. The first growing period lasted from RCP1 to RCP2 with $Ext_1$ extension and $T_1$ duration, and the second growing period lasted from RCP2 to RCP3 with $Ext_2$ extension and $T_2$ duration. Different cultures showed different size control mechanisms.

In the cases of the wild-type haploid and diploid cells, I found negative correlation between the above mentioned growth parameters in the first growth phase, however the duration of the second growth phase and extension during it was independent of $L_{RCP1}$. This suggests that size control operates before RCP2, at mid G2 in these cells (Figure 4).

Negative correlations were also found between both $Ext_1$ and $L_{RCP1}$, and $T_1$ and $L_{RCP1}$, but no correlation was observed in the second growth phase in the case of the wee1Δ mutant. This suggests that size control acts also before RCP2, but due to the differences in the cell cycle phases between wild-type and wee1Δ mutant cells, this means that size control operates in G1 phase here (Figure 4).

In the case of the cdc2-3w mutant cells, weak negative correlations were found between the growth parameters in both growth phases. This suggests that size control either overlaps with RCP2 or it operates both in G1 and G2 phases.

In the cdc2-3w cdc25Δ double and cdc2-3w cdc25Δ pyp3Δ triple mutant cells, the first growth phase showed no correlation between the growth parameters, however negative correlation was found in the second growth phase between both $Ext_2$ and $L_{RCP1}$, and $T_2$
and \( L_{RCP1} \) which suggests that in these cells size control operates after RCP2, i.e. at the end of G2 phase contrary to wild-type cells (Figure 4).

![Figure 4](image1.png)

*Figure 4. Determination of the position of the size control in different fission yeast cultures [4.].*

The late G2 size control observed in the \( cdc2-3w \ cdc25\Delta \) double and \( cdc2-3w \ cdc25\Delta \ pyp3\Delta \) triple mutants was previously known to operate only in these cells. However, a question may arise whether this is a different mechanism or the mid G2 size control is shifted to late G2? To test these hypotheses, further regression analyses were performed, i.e. \( Ext_2 \) and \( T_2 \) were plotted versus \( L_{RCP2} \). Negative correlations were found in all the six strains between these parameters, which suggests that a size compensation occurs in every analysed strain at late G2 (before mitosis). This size control mechanism is probably obscured when regression analyses are based on \( L_{RCP1} \) as reference (Figure 5).

![Figure 5](image2.png)

*Figure 5. The late G2 size control is general in the analysed fission yeast strains [4.].*

The above mentioned results suggest that three different size control mechanisms operate in the fission yeast cell cycle, and these mechanisms can be either operating, or not operating or cryptic, depending on the cells genotype (Figure 6).

It was shown earlier by Fantes in the *cdc2-33* induction synchrony culture, that size control in extremely large cells is abolished. During my PhD work, I have analysed an induction synchrony culture of the same *cdc2-33* mutant as Fantes, but with more precise methods at a higher magnification. In cells shorter than BL = 11.5 μm negative correlation was found between the cycle time and birth length. However, in cells larger than 11.5 μm at birth, the slope of the regression line is close to zero, but it is still significant (Figure 7).

Similar results were obtained when length extension during the cycle was plotted versus birth length. This means that size control acts only in cells being smaller at birth than a critical value, and in cells larger than 11.5 μm the size control is cryptic (the size control ceases in these cells) (Figure 7). If cells are larger at birth than the critical size, then they have very short cycles (also called the minimal cycle time) and they tend to the normal size range during some consecutive generations.

It was shown earlier in budding yeast (*Saccharomyces cerevisiae*) that the growth rate in G1 phase has a direct effect on the cell size at G1/S transition, the point where size control operates in these model organisms. To test whether a similar effect exists in

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fission yeast, first I plotted the growth rate during the growth phase versus the cell size at the start of the growth phase. No correlation was found in four strains, however, in two cases positive correlations were found (WT diploid, cdc2-3w mutant), although this may be the result of the broader size range in these strains.

Then I performed regression analyses, the duration of the growth phase (T), length extension during it (Ext) and length at the end of the growth phase (L_{RCP3}) were all plotted versus growth rate (v) (Figure 8).

**WT**

![Graphs showing the effect of growth rate on different growth parameters in wild-type cells.](image-url)

*Figure 8. The effect of growth rate on different growth parameters in wild-type cells.*
No correlation was found between T and growth rate in five cases (Figure 8.), and positive correlation was found only in the case of the *wee1Δ* mutant, however it was barely significant (*p* = 0.034). Positive correlations were found in all the six strains between both Ext and the growth rate, and also between L-RCp3 and growth rate. This suggests that growth rate has an effect on cell size in fission yeast, so it might be an evolutionary conserved mechanism (Figure 8.).

### 4.3 Phylogenetic analyses

To perform the phylogenetic analysis of some cell wall synthase and hydrolase proteins, first I downloaded the related sequences by searching with the fission yeast proteins' amino acid sequences. In the Taphrinomycotina subphylum, all the related sequences were found in the *Schizosaccharomyces* genus, however in other species no or less homologs were found. In the Saccharomycotina subphylum, neither α-glucan synthase nor α-glucanase homolog was found in any species, probably due to the differences in cell wall composition. In the Pezizomycotina subphylum, several species had 5-6 different α-glucanase and 2-3 α-glucan synthase homolog proteins, and similar results were obtained in the Basidiomycota phylum. After the analysis of the hits' domain structures, several multiple alignment methods and tree constructing strategies were applied both using the full proteins’ and the domains’ sequences.

*Figure 9. Phylogenetic tree of the fission yeast *Agn1* homologous proteins’ GH71 domain constructed by the Bayesian method (WAG+G+I evolution model).*
According to the phylogenetic trees, the fission yeast proteins are potentially more related to the Basidiomycota enzymes than to the Pezizomycotina proteins (Figure 9). It is interesting, because Pezizomycotina species are more related to fission yeasts, however the cell wall composition of *Schizosaccharomyces pombe* is more similar to the Basidiomycota species cell wall than to that of the filamentous ascomycetes.20

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5 Theses

1. In steady-state and induction synchrony fission yeast cultures I have shown, that length growth of the cells is heterogeneous, i.e. the cell growth could not be described by one universal function. Around half – two third of the cells followed a bilinear pattern (two linear segments separated by a breakpoint) and linearly growing cells were also frequent. By contrast, exponential growth patterns were found only in few cases. About half of the bilinearly growing cells had a smooth transition around the rate change point. I also proved that the Akaike Information Criterion is the most appropriate to distinguish among rival models. [1., 3., 4.]

2. I have shown that all the seven hypothetical average cells constructed from the steady-state cultures follow a bilinear length growth pattern. [1., 4., 5.]

3. I have analysed the size control mechanism responsible for the size homeostasis in eight different fission yeast strains at a higher magnification than used in the earlier studies, and by applying sophisticated statistical methods.
   a. I have determined the position of the size control in wild-type and different cell cycle mutant cells. [4.]
   b. I have determined the critical birth length above which the size control ceases (becomes cryptic). [5.]
   c. I have proved that the growth rate of the cell has a direct effect both on the length extension and on the final cell length reached. [4.]

4. I have shown the existence of a size control at the end of the G2 phase, before mitotic onset in every analysed strain. This mechanism was previously known only in some mutants. I have proved that three different size control mechanisms are operating in the fission yeast cell cycle. The G1, the mid G2 and the late G2 size controls can be either operating, or not operating or cryptic, depending on the cells’ genotype. [4., 5.]

5. I have constructed the phylogenetic trees of the α-glucanase and α-glucan synthase proteins among Fungi. These trees revealed that these fission yeast proteins are more closely related to the Basidiomycota ones than to the filamentous Ascomycota enzymes, although the latter ones are taxonomically more related to fission yeast. [2.]
6 Publications

6.1 Publications related to the thesis


6.2 Other publications


6.3 Oral presentations and posters


Sveiczer, Á., Horváth, A., Rácz-Mónus, A., Buchwald, P.: Cell length growth in fission yeast: an analysis of its bilinear character and the nature of its rate change transition. 26th International Conference on Yeast Genetics and Molecular Biology, Frankfurt, Germany, 29 August - 3 September, 2013 (oral presentation)

Horváth, A., Sipiczki, M., Sveiczer, Á.: Phylogenetic analysis of the fission yeast cell separation protein, the endo-(1,3)-α-glucanase Agn1p. 26th International Conference on Yeast Genetics and Molecular Biology, Frankfurt, Germany, 29 Aug - 3 Sept, 2013 (poster)

Horváth, A., Rácz-Mónus, A., Buchwald, P., Sveiczer, Á.: Cell length growth in fission yeast is bilinear and tends to have a smooth transition segment. 4th Central European Forum for Microbiology, Keszthely, Hungary, 16-18 October, 2013 (oral presentation)

Mészáros, Cs., Horváth, A., Sipiczki, M., Sveiczer, Á.: Phylogenetic analysis of the fission yeast cell separation gene, sep15/med8. 4th Central European Forum for Microbiology, Keszthely, Hungary, 16-18 October, 2013 (poster)


Horváth, A. Sveiczer, Á.: Cell length growth patterns in fission yeast reveal a novel size control mechanism operating in late G2 phase. 16th European Cell Cycle Workshop and EMBO Workshop on the Cell Cycle, Budapest, Hungary, 4-7 September, 2015 (poster)