EPIGENETIC SIGNIFICANCE OF CHROMATIN STRUCTURE IN CELLULAR AGEING

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ABSTRACT

Ageing is a process characterized by accumulation of structural and metabolic aberrations which gradually reduce resistance of cells to internal and external stress conditions.

Chromatin is a DNA-protein complex that represents the compaction of DNA in the eukaryotic nucleus and is the platform for all epigenetic processes in the cell. There are lots of data showing the basic epigenetic role of chromatin, especially at its higher levels of compaction in cellular ageing.

In the current experiments we have followed the role of the linker histone H1 and Arp4p, which is a component of three chromatin remodeling complexes, in chromatin dynamics during ageing. We have observed decelerated cellular growth and changes associated with cellular morphology of the studied mutants in the time course of ageing.

Keywords: *chromatin remodeling complex, linker histone, Arp4, ageing*

I. Introduction

Ageing is the certain fate of all living forms on this planet. It is a fascinating phenomenon that has long been the target of excessive scientific research with the main aim to comprehend the nature of this process and to find a way to hinder its manifestation on the organisms. Either way ageing has proven to be an ineluctable obstacle for humankind. Molecular biology is one of the scientific fields that stands on the forefront of studying the ageing process and the general mechanisms that reinforce its effects. Modern-day science has the necessary tools for studying ageing and has the potential to make the required informative and plausible hypothesis on the subject. The time has come for a more decisive push in the field of ageing research and we believe that the gold standard in ageing-related research is none other than chromatin itself. We postulate that chromatin research is the way to go in order to delve even deeper into ageing-related research. There are many authors studying ageing from a point of view targeting only the intrinsic features of ageing like genomic instability, telomere shortening, mitochondrial dysfunction and stem cell exhaustion (López-Otín et al., 2013) but few have been focused on bridging chromatin dynamics to ageing. Chromatin is a highly dynamic and structured nucleoprotein complex of DNA and histones. Chromatin architecture provides a level of gene regulation through the formation of chromatin domains. These domains are evident at the level of local chromatin structure where transcriptionally active genes are associated with "open" and acetylated chromatin turning them on, whereas silent genes are embedded in compacted chromatin which subsequently turns them "off" (Dillon and Sabbattini, 2000). In order for a given gene or a set of genes to be turned ''on'' or ''off" DNA must be in a conformation which allows the molecular machinery of the cell to make contacts with it. This is made possible through the remodeling of chromatin by chromatin remodelers. The last represent protein complexes that regulate chromatin accessibility and thus control DNA-dependent processes (Langst and Manelyte, 2015). Chromatin remodeling is a complex process that requires energy and only by the ability of chromatin remodelers to choose in a highly refined manner their chromatin counterparts these specific remodeling functions are exerted correctly and at the right place (Georgieva et al., 2015). We have demonstrated in previous experiments the dramatic effects that

the abated functions of remodeling complexes exert in yeast cells (Georgieva et al., 2008). Through the introduction of a point mutation in (*ARP4)* gene that codes for Arp4p, which is an important component of three chromatin remodeling complexes in yeast: INO80, NuA4 and SWR1, we have obtained *arp4* mutants and furthermore double mutants which simultaneously lack the gene for the linker histone. Using these mutants we have proven beyond doubt that chromatin structure is maintained by the fine contacts between the linker histones and chromatin remodeling complexes (Georgieva et al., 2008; Georgieva et al., 2015). In an effort to open a new frontier in ageing-related research we have established experiments with the purpose of studying chromatin dynamics through the use of specific *Saccharomyces cerevisiae* mutants. The specificity of these strains is derived from the fact that each strain has a mutation that affects in general its chromatin structure and encourages aberrant morphological phenotype and a predisposition to premature ageing.

Here, we present our results showing that three *S. cerevisiae* chromatin mutants exhibit accelerated ageing during the time of their cultivation. This paper presents collected data acquired during the course of our experiments with these particular yeast chromatin mutants. *S. cerevisiae* fits perfectly in our studies by presenting the advantage to exhibit replicative lifespan and chronological lifespan (Fabrizio et al., 2004). Furthermore given the high degree of similarity between the yeast genes and their human counterparts it is reasonable to say that we can exploit the budding yeast in order to bridge our findings to human disease, severe pathological conditions and especially ageing (Corte-Real and Madeo, 2013).We believe that due to the altered and reformed higher-order chromatin compaction because of the mutations induced in the linker histone and in Arp4p these cells faster lose their morphological tractability and survive worse in comparison to the wild type progenitor strain.

II. Materials and Methods

1. Yeast strains used in the current research:

In the current experiments we have used four strains of *Saccharomyces cerevisiae*: a wild type, Δ*H1, arp4* and double mutant *arp4* Δ*H1* with genotypes listed in Table 1.

Table 1. Genotypes of the studied yeast strains.

WT (wild type)	*MAT*a *his4-912δ-ADE2 his4-912δ lys2-128δ can1 trp1 ura3 ACT3*
Δ*H1* (without the gene for the linker histone)	*MAT*a *his4-912δ-ADE2 his4-912δ lys2-128δ can1 trp1 ura3 act3 ypl127C::K.L.URA3*
arp4 (with a point mutation in the *ARP4* gene)	*MAT*a *his4-912δ-ADE2 lys2-128δ can1 leu2 trp1 ura3 act3-ts26*
arp4 Δ*H1* (double mutant, with a point mutation in *ARP4* gene and without the gene for the linker histone)	*MAT*a *his4-912δ-ADE2 lys2-128δ can1 leu2 trp1 ura3 act3-ts26 ypl127C::K.L.URA3*

2. Cultivation of cells and assessment of cellular growth:

Yeast cells have been cultivated in minimal media (2% Dextrose and 1.7% Yeast Nitrogen Base - YNB), supplemented with all necessary amino acids according to the auxotrophic mutations of the cells. Cultivation was in a water-bath shaker at 30°C by constant rotation. At three fixed time points - 6th hour, 48th hour and at day 5th the optical density (OD_{600}) of the cultures has been measured.

3. Microscopic analysis of cellular morphology:

Cellular morphology of the ageing cells was assessed by preparation of temporary microscopic samples and subsequent observation under the light microscope Leitz VARIO

ORTHOMAT 2 at magnification of 500. Representative images have been bright-contrasted on PhotoShop CS6.1.

4. Data analysis and statistical elaboration of the obtained results:

Data on the graphs with the cellular growth represent the MEAN ± STDV values taken and averaged from three repeated experiments.

III. Results and Discussion

1. The three chromatin mutants have slower cellular growth

Ageing has long been puzzling scientists, at one hand with its complexity and on the other, with its highly individual performance in different organisms even in different individuals. And though, the process involves similar traits and abnormalities (López-Otín et al., 2013) all of them happen at different stages of an organism's lifespan which challenges ageing-related research to set all of its efforts into finding the key mechanism that triggers the ageing onset. This requires the use of the most suitable, less complicated and highly genetically flexible model organism which in the field of ageing research has long ago been found in the yeast *S. cerevisiae* (Breitenbach, 2012; Denoth Lippuner et al., 2014).

Four yeast *S. cerevisiae* strains were used in the current work - WT (wild type) which we have used as a control and three yeast chromatin mutants (Δ*H1, arp4, arp4*Δ*H1)*. Δ*H1* is a mutant that lacks the gene for the linker histone (Georgieva et al., 2012), *arp4* mutant bears a point-mutation in *ARP4* gene which codes for an essential subunit of three chromatin remodeling complexes in yeast (Georgieva et al., 2008; Harata et al., 1999; Harata et al., 2002) and the double mutant *arp4*Δ*H1* which we have designed and created to simultaneously lack H1 and to bear the point mutation in *ARP4 (Georgieva et al., 2015)*.

Cellular growth was assessed by measuring the optical density of the yeast cultures at 600 nm wavelength (OD_{600}) at three time points in the time course of their chronological lifespan (Longo and Fabrizio, 2012; Uzunova et al., 2013). Cells were cultivated in minimal media at optimal conditions and at 6th hour, 48th hour and finally at day 5th aliquots were taken and OD_{600} was measured. Results are presented on Figure 1.

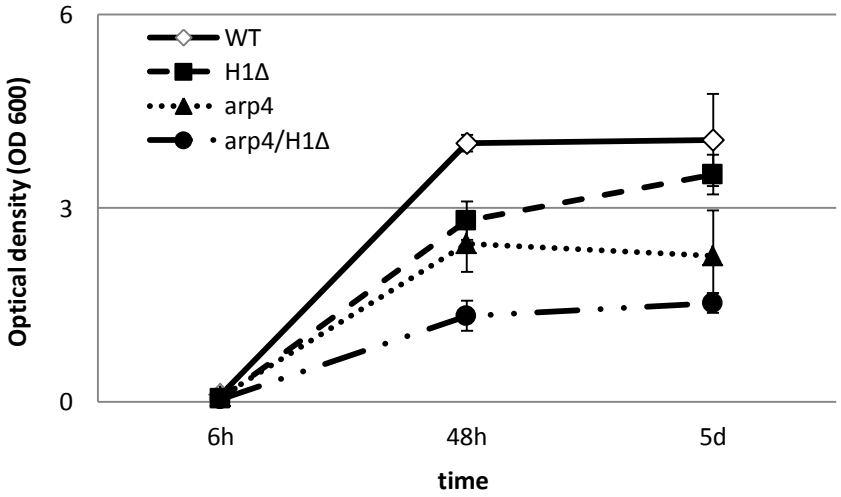

Figure 1. Chronological lifespan of the four yeast strains measured spectrophotometrically at 600 nm wavelength.

Results are obtained from three repetitions of the experiment and values presented are the MEAN values of OD_{600} for each yeast strain at a given time point ± STDV.

We have observed that the three chromatin mutants have decelerated cellular growth in comparison to the wild type. The growth of the double mutant was the most severely affected by the two mutations. Its population of cells was growing more than 70% slower than the WT. During the

time of the experiment none of the three chromatin mutants was able to recuperate its cellular growth and none ever reached the OD_{600} of the control wild type.

2. The three yeast chromatin mutants are unable to maintain normal cellular morphology

One of the hallmarks of ageing is changed cellular morphology which some authors believe is due to altered gene expression, accumulation of misfolded proteins and total inability of the aged cells to control their growth (Burtner and Kennedy, 2010; Hughes and Gottschling, 2012; Laun et al., 2001). In order to study the morphology of the three chromatin mutants we have cultivated cells at optimal conditions for a period of five days. Temporary microscopic slides for light microscopic observations have been prepared on the 6th hour of their cultivation and at day 5th.

The idea was to observe and to compare the cellular morphology of Δ*H1, arp4* and *arp4*Δ*H1* mutant cells with the control. Moreover, we also aimed to compare the young cells of a strain with its old cells and with the old cells of the other mutants.

Representative images of cells of the three yeast mutant strains are shown on Figure 2. We have observed that all three mutants have altered cellular morphology even at the 6th hour of their cultivation. Notably, these cellular abnormalities became more and more explicit with the ageing of the culture (compare young cells with old and WT with the three mutants).

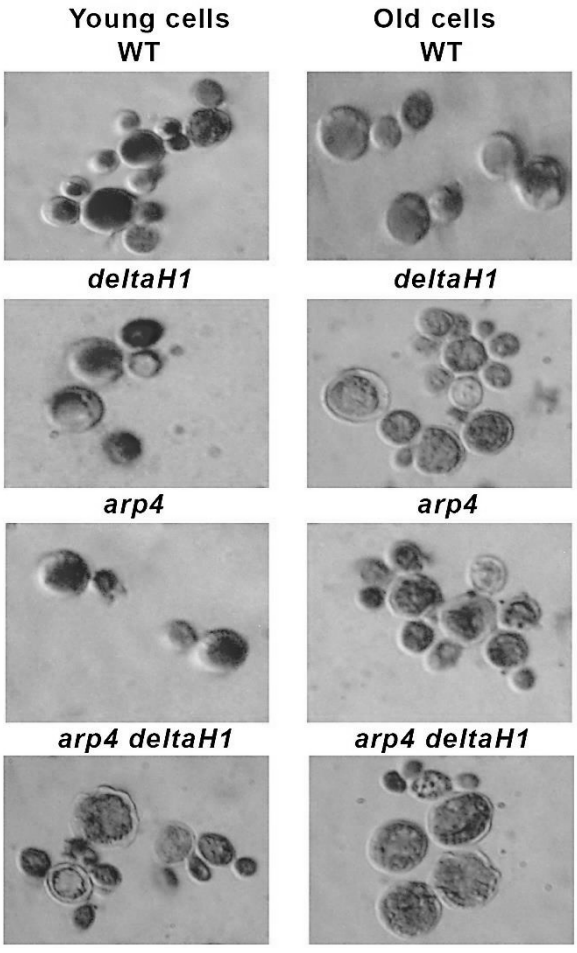

Figure 2. Representative images of WT, Δ*H1, arp4* and *arp4*Δ*H1* yeast cells in the time course of their chronological lifespan.

The cells with the most obvious morphological aberrations were again the double mutant cells. *arp4*Δ*H1*double mutant strain was composed of big, swollen and highly heterogeneous populations of cells even on day first of their lifespan. These aberrations became more and more engraved with ageing (Figure 1, compare *arp4*Δ*H1* young cells with the old cells). Previous studies have demonstrated that these cells possess totally distorted chromatin organization (Georgieva et al., 2015) which proved to be dependent on the abolished natural interaction between the linker histone and Arp4p.

We believe that chromatin and its alterations in the double mutant inflict severe genome rearrangements in these cells which result not only in a sluggish cellular growth but also in apparent morphological anomalies which increase proportionally with the increase of the age of the culture.

IV. Conclusion

The characterization of these three mutants in chromatin structure allowed us to conclude that chromatin is the key mechanism for the fine regulation the activity of the cellular genome and the major weapon for maintaining cellular growth and cellular morphology. This regulation could be easily broken if some of the proteins involved in these mechanisms, in our case H1 and Arp4p, are affected. Regarding the three chromatin mutants used in our research the result of such mutations could not necessarily be fatal for the cells in short

periods of time but could lead to worsening of cellular propagation and explicitly changed cellular morphology with the ageing. The last prompts features of accelerating ageing due to altered chromatin organization and thus makes yeast cells as a brilliant model not only for basic ageing research but also for detailed studies of age-associated human diseases.

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