On The Origin of Ageing

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On The Origin of Ageing

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Abstract

Background
Previously we have shown that the transcription profiles of a small group of functionally related genes are not only heritable but also demonstrate specific changes during ageing. In this paper a new epigenetic theory on ageing is formulated to explain these findings and a method is developed to test this hypothesis. A database with the expression of 33 proteasomal genes in the rectus abdominis from 25 females, differing in age from 29 to 81 years, was analyzed.

Results
Four interrelated parameters reflecting the ageing process were identified: 1) “age effect 1” (based on age-related increase or decrease in expression level), 2) “age effect 2” (based on deviations from the previous age relation), 3) “oscillation index 1”, reflecting a lifelong oscillation in age effect 1 and 4) “oscillation index 2”, reflecting a lifelong oscillation in age effect 2. Parameters of chromosomal architecture (chromosome length, total number of cytogenetic bands, cytogenetic location, number of bands to centromere and number of bands to telomere) correlate with the age-related parameters which reveals an involvement of nuclear architecture in ageing. Regression lines between age and architecture-based expression characteristics indicate that the chromosomal architecture steers ageing and that the physiological age of a tissue can be determined using any group of related genes.

Conclusion
If confirmed further, this epigenetic theory on ageing could alter our current views on ageing.

Introduction
Over the years a gradual shift in our views on ageing occurred. For the greater part of the previous century there was a search for one underlying cause of ageing. This led to the formulation of more than 300 hypotheses (Vina J et al., 2007). So far all theories have failed to present the clue of why ageing occurs (Semsei I, 2000) and gradually ageing became seen as a multi-causal process instead of originating from a single cause (Kowald A & Kirkwood TBL, 1996; Weinert BT & Timiras PS, 2003). Nowadays it is generally believed that the cause of ageing is understood and that ageing is caused by the failure of many maintenance mechanisms (Hayflick L, 2007a; Holliday R, 2006) which lead to the accumulation of random molecular damage (Hayflick L, 2007b; Kirkwood TBL, 2008b). However this latter view on ageing still leads to a number of uncomfortable questions that cannot be accommodated easily. First in this view ageing is essentially a process of accumulation of deleterious events and it is a question how to reconcile this with the orderly process of “normal ageing” that we all know. It can be assumed that compensatory mechanisms exist that lead to a new steady state when accumulation of damage reaches a certain level (Toussaint O et al., 2002) but such compensatory mechanisms are subject to accumulation of damage as well. Thus in this view causes of normal ageing and age-associated diseases do overlap and it is not clarified why ageing appears to be an orderly process. Although conceptually normal ageing can be described as the response to accumulated damage which leads to alterations in homeostasis and age associated disease can be described as a failure of this adaptive mechanism, it seems to remain an unachievable task to describe which changes are deleterious and which normal. Secondly the hypothesis cannot explain why the life-span can be manipulated to a large extent and sometimes with a major effect of only a few genes which indicates that only a small number of processes are causal to all manifestations of ageing (Hekimi S, 2006).

Usually ageing theories are subdivided into two classes: program theories in which ageing is driven by genes and error theories in which ageing results from stochastic events leading to loss of molecular fidelity. Usually it is supposed that an ageing theory has to belong to one of these two classes (Hayflick L, 2007a). However this is not necessarily true: it has been stated that any organized structure that is not in thermodynamic equilibrium will gradually become disorganized and will lead to ageing although it is an enigma which change(s) would dominate the ageing process (Strehler BL, 1986). Use of this thermodynamic view point could lead to a general description system that transcends the program-related and damage-related considerations...
on ageing (Toussaint O et al., 2002). It has been argued that the organization of the genome must play a role in ageing and that the spontaneous organization of complex systems must be involved. However it is a mystery which cellular structure would be basically involved (Macieira-Coelho A, 1997). In a foregoing paper it has already been suggested that ageing results as a consequence of fundamental laws associated with complex systems and thus not by regulatory responses of genes nor by accumulation of damage (Simons JWIM, 2007). In that paper it was thought that in the absence of ageing random heritable drifts in expression levels would occur and that this would lead to destabilization of the whole transcription machinery. It was supposed that this destabilization is prevented by ageing through channeling the heritable drift in expression level into one direction. In this view ageing is the cornerstone that allows the existence of living organisms. In the current paper it is tried to develop this view further into an ageing theory and to provide a method to test this hypothesis. Preferably an ageing theory should have a clear core on the ageing process itself (Lapointe J & HekimiS, 2009).

**Our core statements are:**

1) Ageing is caused by an inevitable increase in entropy in a structural part of the cell, probably the nucleus, not based on damage nor driven by specific genes but based on this structure itself; thus ageing starts with conception.

2) Ageing is epigenetic. This statement results from the successful cloning of animals by nuclear transfer. The fact that deprogramming of somatic cells leads to healthy offspring suggests that many somatic cells do not suffer from a genetic load of mutated DNA and thus that epigenetic changes are involved in ageing.

3) Ageing prevents random heritable changes in cell functioning by canalizing heritable changes into one direction.

4) Since this ageing process is so inherent to the living system it follows that ageing can be investigated by studying he expression in any subset of genes and in a single tissue.

5) Moreover the theory suggests that all existing theories on ageing that are related to damage could be considered as age-associated and not as causal to ageing.

This paper aims to establish whether experimental evidence for this theory can be found. The genes studied are those that code for the proteasome. They were chosen since these genes code for a well preserved, rather simple and well-known structure, present in all cells but otherwise this choice was arbitrary. Although changes in proteasome function and structure in relation to ageing haven been observed no conclusive answer yet exists on the changes in transcription and translation of the proteasomal subunits in relation to ageing (Bulteau AL et al., 2000; Carrard G et al., 2002; Stolzing A & Grune T, 2001). Further it should be kept in mind that in our approach changes eventually found to relate with ageing cannot be due to random mutational events since the target (33 genes per cell) is too small to lead to appreciable effects of mutation. Previous findings.

1) Existence of hereditary profiles in gene expression The method used consisted out of the determination of the degree of deviation from the average expression profile of the proteasomal genes. The transcription profiles of the genes of the 20S proteasome appear to be very heterogeneous, not only for different tissues but also within tissues. However homogeneity of transcription profiles was observed for cell populations of common descent: similar transcription profiles were found for clonal derivatives of tissues e.g., for tumors derived from their normal tissue and for a metastasis derived from a tumor. Thus the transcription profiles differ widely but the transcription profiles themselves appear hereditary. Further characteristics of these transcription profiles are:

- Deviations from the mean transcription profile appear epigenetic in origin and unrelated to mutations and aneuploidy.

- The deviations of individual genes from the mean transcription profile are independent and point to the presence of a structural network in gene expression.

- The genes do not participate equally in the deviations, some genes are more variable in expression than others; this does not relate to the degree of expression of the genes.

- The variation in transcription profiles is larger for tumor tissues than for normal tissues. These findings have been published before (Simons JWIM, 2006).

2) Age related variation in gene expression In our two last papers evidence was found for age related changes in the expression of the proteasomal genes (Simons JWIM, 2007; Simons JWIM, 2009). The method used consisted again in the determination of the degree of deviation from the average expression profile of the genes coding for the 26S proteasome. The degree of deviation was described by a “deviation index”: the standard deviation of the expression levels. A microarray database derived from 90 human donors aged between 22 and 87 years indicated that increase
in donor age correlated with a decrease in deviation index and that the degree of decrease was gender-specific (Simons JWIM, 2007). For that database 25 different tissues were used (Shyamsundar R et al., 2006). Another database derived from only two types of human tissues (Zahn JM et al., 2006) also revealed the existence of age specific changes in the expression of the proteosomal genes (Simons JWIM, 2009). Also tissue- and gender-specificity was observed. Further analysis in both databases revealed heterogeneity among probes in their contributions to these age effects.

In the present paper the age associated changes in the muscle database are further analyzed, specifically to establish whether these changes could be related to the nuclear architecture and whether ageing goes with unidirectional changes in gene expression. The analysis pertains only to the data for the musculus abdominis of the 25 females.

Results

Identification of Age-related parameters

The first step of the analysis consists in the identification of a number of parameters that reflect the ageing process. The course of this analysis is given in Illustration 4: theoretically two age effects can be distinguished: firstly an age dependent increase or decrease in the expression level of a gene (Age effect 1) and secondly an age dependent increase or decrease in the deviations from these regression lines between age and expression level (Age effect 2). Two further parameters reflect the degree and direction of eventual non-linearity of these two types of regression. The relation of age with expression level of individual probes (expression-data shown in “Additional file”, database AA) results in 62 regression lines, 25 with a negative slope and 37 with a positive slope. This difference is not significant (p = 0,127) but if there is a relation between age and expression of the probes, the probes can be divided into 2 types: those that tend to increase in expression with age (further referred to as "increase-probes") and those that tend to decrease in expression with age (decrease-probes). Subsequently it was tested whether these regression lines deviate from linearity. The method is shown in illustration 5: the expressions are divided into 3 age groups: 1) young: 29 – 41 years, 2) middle aged: 43 – 67 years and 3) old: 73 – 81 years and it was tested whether the negative and positive deviations from the linear regression line occur at random by summing up the data as shown in Illustration 5. This procedure leads to two values per regression line that should be equal when the lines are linear. A contingency table with the two values from all 62 probes proves that non-linearity is present (p = 0,0018), indicating that with age an oscillation is occurring in the expression of the genes. Moreover two types of oscillation are occurring: one with increase in expression for the middle-aged group and one with decrease in expression, the first type being more frequent (41 versus 21, p = 0,011). Therefore, instead of a linear regression model, also a polynomial regression model was applied in which the quadratic term accounts for the non-linearity. This quadratic term varies from -0,624 to +0,640. Illustration 6 shows the relation between age and this non-linearity in expression. For this illustration the probes were divided into three groups: group A with 20 probes varying in quadratic term from -0,624 to -0,196, group B with 22 probes varying in index from -0,170 to 0,063 and group C with 20 probes varying in index from 0,074 to 0,640. For each probe the mean expression level per group was determined and for each group the relation between age and these mean expression levels was established (Illustration 6). This illustration shows that there is an oscillation in expression during the lifespan for at least a part of the probes and indicates that the direction of this oscillation varies per probe. Therefore the quadratic term of the polynomial regression serves as an index for the oscillation and is further referred to as “Oscillation index 1”.

The next step in the analysis investigates whether an Age effect 2 occurs: a relation of age with the deviations from the regression line between age and probe expression level. Since at least a part of the latter regression lines proved to be non-linear, deviations from the polynomial regression line were used. These deviations are also shown in the file with “Additional file”. In order to be able to calculate a regression line between age and degree of deviation, negative deviations were transformed into positive values. The p-values of the 62 regression lines between age and absolute deviations differ widely from 0,0008 to 0,989. Of the 62 regression lines, 38 have a negative slope and 24 a positive slope. This difference is not significant (p = 0,075) but large enough to suggest that generally the deviations might decrease with age.

To test whether an oscillation in deviation from this regression line occurs during ageing, the method for testing linearity, shown in illustration 5, was applied to the regression lines of age with degree of absolute deviation also. The same three age groups were used as before. The contingency table with the two values for all 62 probes shows that Age effect 2 is non linear also (p = 0,00025), indicating that with age an
oscillation is occurring in the deviations from the regression lines. Moreover two types of oscillation are occurring: one with increase in deviations for the middle-aged group and one with decrease in deviation, the second type being more frequent (49 versus 13, \( p = 4.8 \times 10^{-6} \)). Again the degree of deviation can be expressed in an index: the coefficient of the quadratic term of the polynomial regression. This index (Oscillation index 2) varies from -0.426 to +0.552. In Illustration 6 the relation between age and deviation is shown. For this illustration the probes were again divided into three groups: group D with 20 probes varying in “Oscillation index 2” from -0.426 to -0.020 group E with 22 probes varying in index from -0.015 to 0.088 and group F with 20 probes varying in index from 0.090 to 0.552. Again it is suggested that lifelong oscillations occur, this time in the deviations from the regression line between age and expression level. All together this analysis indicates that age effects do occur and that the probes can be arranged in a continuum for these effects of age. First age can go with either an increase or a decrease in expression (Age effect 1) and this alteration is not completely linear with age (Oscillation index 1); secondly the degree of deviation from this regression line also increases or decreases with age (Age effect 2) and these alterations with age are also not completely linear (Oscillation index 2).The values of the four parameters for each probe are shown in Illustration 1. The relation between the four age parameters (Age effect 1 and 2 and Oscillation index 1 and 2) is shown in a correlation matrix in Illustration 2. All correlations are significant suggesting that the four parameters reflect one age related phenomenon. When separate correlation matrices are determined for the probes that increase in expression with age and for the probes that decrease in expression with age a difference is indicated between these two groups: in the group of decrease-probes Age effect 1 appears to be the most important while in the group of increase-probes Oscillation index 2 is the most important (Illustration 2). This indicates that the two groups might be basically different.

**Effects of age and chromosomal architecture:**

The Epigenetic Theory On Ageing postulates that ageing occurs because of changes in the nucleus. Therefore it was investigated whether the four age-associated parameters correlate with some parameters of chromosomal architecture. As parameters of chromosomal architecture were chosen: 1) length of the chromosome (in millions of base pairs) on which a gene is located, 2) total number of cytogenetic bands in the chromosome on which a gene is located, 3) cytogenetic location of the gene, 4) distance of the gene to the centromere in number of cytogenetic bands and 5) distance of the gene to the telomere of the chromosome arm in which the gene is located, also in number of cytogenetic bands. Initially the analysis was performed on the 33 genes, in case a gene is represented by more than one probe, the mean values of the parameters were taken. The correlations between parameters of chromosomal architecture and parameters of ageing are shown in illustration 3A. Apparently this approach hit the bull’s eye: 6 of the 20 correlations were significant: 3 for Age effect 1, 2 for Age effect 2 and 1 for Oscillation index 1. Chromosome length and total number of cytogenetic bands in the chromosome correlated both with Age effect 1 and with Age effect 2 (\( p = 0.014 \); \( p = 0.017 \); \( p = 0.002 \) and \( p = 0.015 \) respectively), suggesting that the chromosomal architecture is a main determinant in age-related changes.

When, instead of using mean values per gene, individual values of the 62 probes were used for the analysis most of the foregoing correlations are less pronounced and less significant in spite of the increased number of data. (Illustration 3B). This suggests heterogeneous age effects for different probes of the same gene. Therefore a similar analysis was performed for the 20 probes that have the most negative values in the age-related parameter and for the 20 probes that have the most positive values (Illustration 3C1 and 3C2). It is indicated that chromosome length is more involved in age-associated changes in a part of the probes while distance to telomere and centromere is more involved in another part of the probes.

Illustration 3 shows that 17 of the 80 correlations between age related parameters and chromosomal architecture related parameters are significant at the 5% level indicating that the presence of significance correlations is not due to the large number of comparisons. Moreover of the corresponding p-values, 56 are smaller than 0.500 and 24 larger indicating the presence of significant correlations (\( p = 0.0017 \)). Thus it must be concluded that the chromosomal architecture is involved in ageing. Determination of physiological age. Since the chromosomal architecture appears involved in ageing it could be possible to link age directly to chromosomal architecture. Therefore, for each library, the relation between chromosome length and Age effect 2 was established. All 62 probes were used for this calculation and each library provides a slope between chromosome length and absolute deviation that is characteristic for the donor of the tissue. All 25 donors together provide the relation between donor age and this characteristic slope (Illustration 7). In
Illustration 7A the regression between age and this characteristic slope is not significant but a tendency is present (p = 0.082). Since previous findings suggested that probes differ in their contribution to the ageing process (Simons JWIM, 2007; Simons JWIM, 2009) and since differences are indicated between increase-probes and decrease-probes (illustrations 2 and 3), the same calculation was performed for two subgroups of probes: one group consisting out of the 20 decrease-probes (decrease in expression with age) and one group consisting out of the 20 increase-probes (increase in expression with age). Illustration 7B shows that the 20 decrease-probes lead to a very significant effect of chromosome size in ageing (p = 0.0021) and this relation with age appears linear. The 20 increase-probes do not show an effect of age (p = 0.573). Illustration 7C shows a similar relation between age and the slope of chromosome size and deviations for the 20 probes with the highest oscillation index2 (p = 0.008). Therefore in principle it seems possible to determine the physiological age of a tissue and thus to identify donors with an aberrant (or variant) ageing process.

Discussion

Support for the epigenetic theory on ageing
The foregoing supports to a large extent the views on ageing formulated in a foregoing paper (Simons JWIM, 2007) and in the introduction of the current paper. A number of parameters reflecting age-related changes in gene expression was found to correlate with parameters of chromosomal architecture. Although only a correlation this indicates that, as predicted by the epigenetic theory on ageing, structural changes in chromosomes are responsible for the ageing process. That these changes are epigenetic (thus not based on damage to the DNA) is indicated by the size of the target: 825 genes (33 genes in 25 individuals). One mutation in this target requires a mutation frequency of 12 x 10^-4 which is already well beyond usual spontaneous mutation frequencies. Therefore the great majority of cells cannot have accumulation of mutational damage in the proteasomal genes. To save the DNA-argument one could argue that the observed changes are a systematic change to random DNA damage elsewhere in the genome. This would require the presence of an elaborate and gene-driven signaling and response system that induces the epigenetic ageing phenotype. This seems extremely unlikely. The study of how the chromosomal and nuclear structure is related to the epigenetic ageing process, although equally enigmatic, seems feasible and more inviting. So far our study only indicates that a lifelong oscillatory process is involved that results in an unidirectional change in the relation between chromosomal structure and gene expression.

The epigenetic theory predicts that epigenetic age-related changes can be detected in the expression of any subset of genes and in any tissue. So far only one subset of genes was investigated in a single tissue and thus a genome wide extrapolation is still highly speculative and requires many more data in order to reveal a basic principle of ageing. However the data obtained thus far with this study support the epigenetic theory on ageing. The epigenetic theory on ageing and current views on ageing.

The current theory on ageing is the network theory. According to this theory ageing is due to the gradual accumulation of un repaired random molecular faults, leading to a mosaic of more and less damaged cells, leading ultimately to a functional decline of cells and system. Since there is a broad range of accumulating stochastic faults in molecules, cells and tissues, understanding of ageing can only be expected by adopting an integrative approach that simultaneously explores the whole network of interactions of age related changes (Kirkwood TBL, 2005; Kirkwood TBL, 2006; Kirkwood TBL, 2008a).

The epigenetic theory is a competing hypothesis. It is not based on the accumulation of errors nor on a gene driven program, instead it is based on the spontaneous increase in entropy of the structure of the cell ultimately leading to impairment of function. Although this possibly relates to a single mechanism it does not exclude that a high level of complexity in ageing still exists since gene transcription and translation depend on a complex interplay of many processes and structures. According to the network theory the lifespan of an organism is in principle indefinite provided that the maintenance mechanisms are efficient enough. According to the epigenetic theory the lifespan is always limited also in the presence of optimal maintenance mechanisms since ageing is intrinsic to the system. Only the rate of ageing appears open to change. Therefore the epigenetic theory explains healthy ageing while the network theory predicts an overlap for the causes of ageing and the causes for age-related diseases and thus has a problem in explaining healthy ageing. In the epigenetic theory there is no overlap between the causative pathways of ageing and age related disease. Therefore according to the epigenetic theory it could be more appropriate to restrict the role of failing maintenance systems to their involvement in age-related disease only.

Testability of the epigenetic theory on Ageing
In contrast to all other views on ageing, it seems relatively easy to test the validity of the epigenetic theory on ageing. To analyze the ageing process further along the lines shown here, this study provides some clues that could be taken into account. First data analysis should be applied to only one tissue type at a time and, if the tissue consists of different types of cells, should be restricted even to one type of cell. Moreover, since gender specific differences are to be expected the analysis has to be restricted to one gender at a time. Secondly the number of libraries to be used should be large. Although this study revealed age effects in only 25 libraries, it is felt that a higher number would be much better and that young and old age should represented more equally. Moreover, since the ageing process is expected to start from conception, also really young donors could be included. Thirdly the number of genes has to be expanded and in view of the possible role of chromosome size in ageing it could be appropriate to choose preferentially genes located on one of the larger chromosomes and on one of the smaller chromosomes. Also the distribution of the locations of the genes along the chromosome could be taken into account for the selection of the genes. Fourthly for a choice of the model organism to be used it is worthwhile to consider the potential advantage of in vitro ageing. The degree of relevance for ageing of the limited life span of cultured cells in vitro has been a topic for debate a long time (Macieira-Coelho A, 2003). Nevertheless, if the epigenetic theory would hold, it can be expected that the basic principle of epigenetic ageing (unidirectional changes in gene expression mechanisms to avoid random drift in gene expression) should be operationally in cultured cells. Then, initially, the model of in vitro ageing has certain advantages over the use of whole organisms for the study of the basic principle of ageing. With cultured cells the collection of materials is much easier and the application of duplicates and longitudinal studies are more feasible. In conclusion, since the results are in line with the core statements of the Epigenetic Theory on Ageing, this theory could, if validated by further research, lead to an alternative approach in ageing research and eventually replace our current views on ageing.

**Methods**

**Experimental procedures:**
A part of the database on human muscle from GEO, GSE5086 was used (Zahn JM et al., 2006). This part of the database consists out of 25 libraries derived from the rectus abdominis of females. The donors varied in age from 29 to 81 years. The transcription profiles of the 33 genes that code for the 26S proteasome were used for the analysis.

In the database 62 probes are available for these 33 genes:
- 1 probe for PSMA4, A6, B3, B6, C1, C3, C4, C5, C6, D2, D3, D7, D8, D14,
- 2 probes for A2, A5, A7, C2, D1, D6, D9, D10, D11, D12, D13,
- 3 probes for A1, A3, B1, B2, B4, D4, D5 and
- 5 probes for B7.

No probe was available for PSMB5.

When more than one probe is available for a particular gene, the gene symbol was extended with alphabetic characters:
- A1a, A1b etc.

The Additional file lists the probe sets and their expression in the 25 libraries:
- The size of the chromosomes and the numbers of cytogenetic bands in the chromosomes were derived from “GeneLoc” ([http://genecards.weizmann.ac.il/geneloc](http://genecards.weizmann.ac.il/geneloc))
- All calculations were performed with XLSTAT

**Additional file**

Supporting Information
The additional file contains 3 databases: 1) database A with the raw expression levels of the 62 probes in the 25 libraries (Zahn JM et al., 2006), 2) database AA in which the mean expression levels of the probes have been transformed to a mean of 1000 and 3) database AB in which for each probe the deviation in expression level is shown from the expected expression level as calculated with the polynomic regression line between age and probe expression level.

**References**

3. Hayflick L (2007a) Biological ageing is no longer an

Acknowledgements

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Illustrations

Illustration 1

Age associated parameters, Age effect 1 is the slope of the regression line between age and expression level; Age effect 2 is the slope between age and the deviations from the previous regression line; Oscillation index 1 reflects the non-linearity of the regression line of Age effect 1 while Oscillation index 2 reflects the non-linearity of the regression line of Age effect 2.
Illustration 2

Correlation between the four age-related parameters (upper triangle) and their significance (lower triangle). Significant values are in bold. Increase-probes and decrease-probes differ with respect to the importance of the age parameters. Age effect 1 appears most important for decrease-probes and Oscillation index 2 appears most important for increase-probes.

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Illustration 3

Significant correlation between parameters of ageing and parameters of chromosomal architecture. Significant values are in red (p

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<th align="left">Table 1: Correlation between parameters related to chromosomal architecture</th>
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<th>Slope</th>
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<th>Oscillation</th>
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<td align="left">Age-related parameters and chromosome length (mils of bp)</td>
<td>effect 1</td>
<td>effect 2</td>
<td>index 1</td>
<td>index 2</td>
</tr>
<tr>
<td align="left">All 62 probes</td>
<td>-0.290</td>
<td>0.181</td>
<td>0.097</td>
<td></td>
</tr>
<tr>
<td align="left">Total number of bands</td>
<td>-0.290</td>
<td>0.222</td>
<td>0.154</td>
<td>0.106</td>
</tr>
<tr>
<td align="left">Cytogenetic location</td>
<td>-0.230</td>
<td>0.062</td>
<td>0.351</td>
<td>0.104</td>
</tr>
<tr>
<td align="left">N o of bands to centromere</td>
<td>-0.154</td>
<td>0.053</td>
<td>0.087</td>
<td></td>
</tr>
<tr>
<td align="left">N o of bands to own telomere</td>
<td>0.021</td>
<td>0.141</td>
<td>0.082</td>
<td>0.108</td>
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</table>

<table>
<thead>
<tr>
<th align="left">Table 1: Correlation between parameters related to chromosomal architecture</th>
<th>Slope</th>
<th>Slope</th>
<th>Oscillation</th>
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</thead>
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<td>effect 1</td>
<td>effect 2</td>
<td>index 1</td>
<td>index 2</td>
</tr>
<tr>
<td align="left">C1: 29 probes most negative in value</td>
<td>-0.670</td>
<td>-0.199</td>
<td>0.636</td>
<td>-0.121</td>
</tr>
<tr>
<td align="left">Total number of bands</td>
<td>-0.499</td>
<td>0.296</td>
<td>0.188</td>
<td>0.015</td>
</tr>
<tr>
<td align="left">Cytogenetic location</td>
<td>-0.243</td>
<td>0.262</td>
<td>0.247</td>
<td>0.330</td>
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<tr>
<td align="left">N o of bands to centromere</td>
<td>-0.121</td>
<td>0.177</td>
<td>0.286</td>
<td>0.309</td>
</tr>
<tr>
<td align="left">N o of bands to own telomere</td>
<td>-0.352</td>
<td>0.117</td>
<td>0.178</td>
<td>-0.374</td>
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</tbody>
</table>

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<td>effect 2</td>
<td>index 1</td>
<td>index 2</td>
</tr>
<tr>
<td align="left">C2: 29 probes most positive in value</td>
<td>0.029</td>
<td>0.110</td>
<td>0.139</td>
<td>0.504</td>
</tr>
<tr>
<td align="left">Total number of bands</td>
<td>-0.600</td>
<td>0.149</td>
<td>0.106</td>
<td>-0.044</td>
</tr>
<tr>
<td align="left">Cytogenetic location</td>
<td>-0.224</td>
<td>0.199</td>
<td>0.045</td>
<td>0.123</td>
</tr>
<tr>
<td align="left">N o of bands to centromere</td>
<td>-0.013</td>
<td>-0.800</td>
<td>-0.091</td>
<td>0.036</td>
</tr>
<tr>
<td align="left">N o of bands to own telomere</td>
<td>0.005</td>
<td>0.034</td>
<td>0.054</td>
<td>0.163</td>
</tr>
</tbody>
</table>
Illustration 4

Outline of analysis of effects of age for an arbitrary probe in the database. Age effect 1: regression line between age and probe expression level, Age effect 2: regression line between age and the absolute deviations seen in the previous regression line. From both regression lines are used: a) slope regression line, b) an index for the eventual non-linearity of the regression line.

Age effect 1
Age with probe expression

- y = 3.855x + 776.6

Age effect 2
Age with absolute deviations

- y = 0.364x + 61.15
Illustration 5

Method used to determine whether an oscillation in gene expression occurs during the lifespan. The data are from probe PSMA4. The number of points in the shaded areas is compared with the number of points in the other areas with chi-square (here \( p = 0.028 \)). As index for the degree of oscillation the coefficient of the quadratic term of the polynomic distribution is used, in this particular case: 0.254
Illustration 6

Life-long oscillation in the expression of genes. 1) regression of age with mean expression level per group for 3 groups with increasing Oscillation index 1: group A (20 probes), group B (22 probes) and group C (20 probes). 2) regression of age with the mean absolute deviation from the first regression line for 3 groups with increasing Oscillation index 2: group D (20 probes), group E (22 probes) and group F (20 probes).
Illustration 7

Age dependence of the slope of the regression between size of the chromosomes and age-parameters. A: slopes calculated for Age effect 2 with all 62 probes (p = 0.082), B: slopes calculated with the 20 decrease-probes only (p = 0.0021), C: slopes calculated for Oscillation index 2 for 20 increase probes (p = 0.008; without outlying value p = 0.02).
Reviews

Review 1

Review Title: An Informative Paper But Not Without Controversies

Posted by Dr. Marios Kyriazis on 07 Dec 2012 11:20:59 AM GMT

What are the main claims of the paper and how important are they?:
This is essentially a study of some epigenetic mechanisms involved in ageing. It is indeed an important subject and one likely to provide clues as to ways for manipulating ageing.

However, in the Abstract conclusion there should be some more details explaining how exactly the epigenetic theory could alter our views on ageing, and what the consequences of it would be.

Yes. This is a new way of seeing ageing, particularly as being distinct from other theories. However, the claim that ageing is due to an inevitable increase in entropy is not, in my view, incompatible with other theories of ageing such as the stochastic damage theories, or even the programmed ageing ones.

Yes. There is extensive and useful literature.

Yes. It would be helpful to expand on this approach from different angles in subsequent papers or research. This will strengthen the author's position.

If a protocol is provided, for example for a randomized controlled trial, are there any important deviations from it? If so, have the authors explained adequately why the deviations occurred?

NA

NA

Not at this stage, but perhaps in separate papers.

Not at this stage, but perhaps in separate papers.

Rating: 6

Comment:
No

Competing interests: No

Invited by the author to make a review on this article?: No

Have you previously published on this or a similar topic?: Yes

References:


Review 2

Review Title: On the origin of ageing

Posted by Prof. Mahadev Murthy on 29 Apr 2012 01:47:19 PM GMT

1 Is the subject of the article within the scope of the subject category? Yes
2 Are the interpretations / conclusions sound and justified by the data? Yes
3 Is this a new and original contribution? Yes
4 Does this paper exemplify an awareness of other research on the topic? Yes
5 Are structure and length satisfactory? Yes
6 Can you suggest brief additions or amendments or an introductory statement that will increase the value of this paper for an international audience? No
7 Can you suggest any reductions in the paper, or deletions of parts? No
8 Is the quality of the diction satisfactory? Yes
9 Are the illustrations and tables necessary and acceptable? Yes
10 Are the references adequate and are they all necessary? Yes
11 Are the keywords and abstract or summary informative? Yes

Rating: 7

Comment: This is an interesting paper, which makes strong statements on ageing. If we are able to understand the physiological ageing, it would change the direction of how we approach ageing theories. There are however indications that the nuclear architecture might be involved in the ageing process. The data from this paper tends to support this theory.

The epigenetic-based changes are beginning to emerge as significant factors in ageing. In addition, these changes also seem to address this issue from an evolutionary perspective. The aspect of heritability of certain genes is also credible despite the complexity associated with the biology of ageing.

The findings support another way of looking at ageing theories. Changes have been found in the proteasome function and structure although no clear conceptual theory has evolved for ageing. The authors make a strong case in support of their findings.

It is a well-written paper and should be accepted. However, there are a few typos/errors in the paper. They should be corrected.

Competing interests: no

Invited by the author to make a review on this article?: Yes

Have you previously published on this or a similar topic?: Yes


Experience and credentials in the specific area of science:
I work in the area and I am interested in understanding the biology of ageing and in identifying gaps that would help us understand physiological ageing. This also helps us decide what we should support in terms of ageing research to advance intervention approaches.

How to cite: Murthy M. On the origin of ageing [Review of the article ‘On The Origin of Ageing’ by
Review 3

Review Title: Good work, but within a questionable paradigm

Posted by Dr. Giacinto Libertini on 17 Dec 2011 03:41:26 PM GMT

<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>Answer</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
<td>11</td>
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</tbody>
</table>

Rating: 6

Comment:
More attention should be given to checking the correct citation of references (e.g. Vina J et al., 2007 or 1999; Lapointe J, Hekimi S, 2009 or 2010?).

Apart from these inaccuracies (which can be easily corrected), the A. maintains that “the cause of ageing is understood and ageing is caused by the failure of many maintenance mechanisms …”. This prejudicial conviction is contradicted by many known data (see Libertini G.. Empirical evidence for various evolutionary hypotheses on species demonstrating increasing mortality with increasing chronological age in the wild, TheScientificWorldJournal, 2008, 8, 183-193): the persistence in supporting certain hypotheses in contrast with known facts can be explained by the predictions expressed in the fundamental work of Kuhn T, 1970 (see Milewski L., The evolution of ageing. Bioscience Horizons, 2010, 3, 77-84).

However, in a theoretical context not yet proven and indeed contradicted by evidence, I consider the work interesting and worth exploring within a different theoretical paradigm.

Competing interests: No

Invited by the author to make a review on this article? : No

Have you previously published on this or a similar topic?: No

Experience and credentials in the specific area of science:
Please, see my personal page (www.r-site.org/ageing) and the site www.programmed-aging.org

How to cite: Libertini G.Good work, but within a questionable paradigm[Review of the article ‘On The Origin of Ageing’ by ].WebmedCentral 1970;2(12):WMCRW001279
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