Correlation of mitochondrial superoxide dismutase and DNA polymerase β in mammalian dermal fibroblasts with species maximal lifespan

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Abstract

Eukaryotic cells have evolved elaborate mechanisms to preserve the fidelity of their genomic material in the face of chronic attack by reactive byproducts of aerobic metabolism. These mechanisms include antioxidant and DNA repair enzymes. Skin fibroblasts of long-lived mammalian species are more resistant to oxidative stress than those of shorter-lived species [Kapahi, P., Boulton, M.E., Kirkwood, T.B., 1999. Positive correlation between mammalian life span and cellular resistance to stress. Free Radic. Biol. Med. 26, 495–500], and we speculated that this is due to greater antioxidant and/or DNA repair capacities in longer-lived species. We tested this hypothesis using dermal fibroblasts from mammalian species with maximum lifespans between 5 and 122 years. The fibroblasts were cultured at either 18 or 3% O2. Of the antioxidant enzymes only manganese superoxide dismutase was found to positively correlate with maximum lifespan (p < 0.01). Oxidative damage to DNA is primarily repaired by the base excision repair (BER) pathway. BER enzyme activities showed either no correlation (apurinic/apyrimidinic endonuclease), or correlated negatively (p < 0.01) with donor species MLS (polymerase β). Standard culture conditions (18% O2) induced both antioxidant and BER enzymes activities, suggesting that the 'normal' cell culture conditions widely employed are inappropriately hyperoxic, which likely confounds the interpretation of studies of cellular oxidative stress responses in culture.

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Keywords: Free radicals; Oxidative stress; Longevity; Reactive oxygen species; Mitochondria; ROS; Superoxide dismutase; BER

1. Introduction

Cellular deterioration resulting from chronic oxygen radical attack of proteins, lipids, and nucleic acids is thought to play an integral role in animal aging (Finkel and Holbrook, 2000; Kregel and Zhang, 2007). A number of studies support the hypothesis that enhanced longevity is associated with increased cellular resistance to oxidative stress. For instance, fibroblasts cultured from the long-lived Snell dwarf mouse are more resistant to various oxidative stressors than their normal heterozygous littermates (Murakami et al., 2003). Similarly, oxidative stress resistance of cultured fibroblasts is strongly correlated with longer lifespan in mammals (Kapahi et al., 1999). Indeed, differences in cellular resistance to oxidative stress appear to underlie the observation that mouse dermal fibroblasts in culture senesce earlier than their human counterparts (Parrinello et al., 2003).

However, the mechanisms underlying these differences in the oxidative stress resistance of cultured cells have not been directly investigated. Eukaryotic cells have elaborate defense systems, including antioxidant enzymes for detoxifying ROS and repair enzymes that remove oxidative damage from DNA. Enhancement of either of these properties could potentially confer greater stress resistance to the cells of longer-lived animals. Hart and Setlow (1974) described a positive correlation between UV-induced repair in skin fibroblasts and mammalian lifespan. Although this study suggests a connection between fibroblast DNA repair capacity and species lifespan, UV-induced lesions are primarily repaired via the nucleotide excision repair (NER) pathway. Oxidative damage leading to DNA base modifications or base loss is repaired primarily by the base excision repair (BER) pathway (Wilson and Bohr, 2007). Consequently, an investigation into the relationship between cellular BER capacity and species lifespan is warranted.

The aim of this study was to investigate the correlation of both enzymatic antioxidant capacity and DNA BER capacity of fibroblasts in culture with mammalian species longevity. To this end, activities of the antioxidant enzymes catalase (CAT) and
glutathione peroxidase (GPx), and levels of the mitochondrial isofrom of superoxide dismutase (MnSOD) were quantified in fibroblasts from eight mammalian species with maximum lifespans (MLS) ranging from 5 to 122.5 years. Similarly, cellular BER capacity was also evaluated by measuring the maximal activities of two enzymes catalyzing critical steps in the BER pathway: apurinic/apyrimidinic endonuclease (APE1) and polymerase β (β-pol).

Typically, mammalian fibroblasts are cultured under conditions in which the oxygen content of the culture medium is unregulated (a standard O2 (stdO2) environment). Oxygen levels will thus equilibrate to approximately 18% O2 in an incubator flushed periodically with CO2 to maintain the latter gas at 5%. This results in a substantially hyperoxic medium (Brown et al., 2007) that appears to impose an oxidative stress on cultured cells. This could obscure between species differences in antioxidant or BER activities. Alternatively, it is also possible that the cells of longer-lived species are better able to initiate an appropriate oxidative stress resistance response, allowing them to better withstand the stdO2 environment. We propagated cells from all species under either a stdO2 or physO2 environment, to investigate innate and oxidative stress-induced activities.

2. Experimental procedures

2.1. Materials

Modified Eagles Medium with Earl salts, t-glutamine and sodium bicarbonate was obtained from Sigma–Aldrich (St. Louis, MO). Penicillin/streptomycin, non-essential amino acids and fetal bovine serum was obtained from Hyclone (Logan, Utah). Oligonucleotides were obtained from The Midland Certified Reagent Co. (Midland, TX). Human apurinic/apyrimidinic (AP) endonuclease 1 (APE1) and human polymerase beta was purchased from Trevigen (Gaithersburg, MD). All other chemicals and purified enzymes were obtained from Sigma–Aldrich (St. Louis, MO). Penicillin/streptomycin, non-essential amino acids and fetal bovine serum was obtained from Hyclone (Logan, Utah). Oligonucleotides were obtained from The Midland Certified Reagent Co. (Midland, TX). Human apurinic/apyrimidinic (AP) endonuclease 1 (APE1) and human polymerase beta was purchased from Trevigen (Gaithersburg, MD). All other chemicals and purified enzymes were obtained either from Sigma–Aldrich (St. Louis) or Fisher Scientific (Mississauga, Canada) unless otherwise stated.

2.2. Cell lines and growth conditions

Table 1

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<th>Repository number</th>
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<th>PDL range</th>
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<td>Homo sapiens</td>
<td>122.5</td>
<td>Adult</td>
<td>Female</td>
<td>16–17</td>
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</tbody>
</table>

2.3. Preparation of whole cell extracts

Whole cell extracts were prepared from eight 100 mm plates of each cell line. A total of three extracts were prepared per cell line. Cells were lysed by incubation for 1 h with periodic sonication (Ultrasonic Inc. Sonicator W-375; setting 3) in 0.5 ml of ice cold lysis buffer (10 mM Tris pH 8.0, 150 mM NaCl, 2 mM EDTA, 2 mM dithiothreitol (DTT), 0.4 mM phenylmethylsulfonylfluoride (PMSF), 40% glycerol and 0.5% NP40). Following incubation, cell lysates were centrifuged at 13,000 × g, 4 °C for 10 min (Fisher Scientific accuSpin™ Micro R). Protein content of the cell lysates was determined by the Bradford method using a BioRad protein assay kit. Lysates were stored at –80 °C.

2.4. Antioxidant enzyme assays

CAT activity was determined by the decrease in the absorbance due to hydrogen peroxide (H2O2), measured at 240 nm and 30 °C on a Varian Cary 300 UV–vis spectrophotometer. The assay buffer contained 25 mM potassium phosphate buffer (pH 7.2) and 0.1 M H2O2. The reaction was initiated by the addition of 20–100 µg protein and absorbance was followed for 4 min.

GPx activity was determined by the decrease in the absorbance of NADPH at 340 nm and 30 °C. The assay buffer contained 50 mM potassium phosphate buffer (pH 7.0), 0.4 mM EDTA, 0.15 mM β-NADPH, 1 U glutathione reductase, 1 mM glutathione, and 2–10 µg protein. The reaction was initiated with the addition of 0.0007% H2O2 and absorbance was followed for 6 min.

2.5. Immunoblotting for MnSOD

Changes in MnSOD levels across species and oxygen tension were determined by Western blot analysis. Equal amounts of whole cell extract (20 µg) were subjected to SDS-PAGE on 5% stacking, 12% resolving and transferred to polyvinylidene difluoride membrane. As a loading/control membrane, membranes were stained with Memcode reversible protein detection as per manufactures instructions (Pierce, Rockford, IL). Western blot analysis was performed using the Odyssey (Li-Cor, Lincoln, NE) detection system as recommended by the manufacture, probing with rabbit antibody raised against a synthetic peptide corresponding to amino acid residues 183–199 of human MnSOD (SS5069, DD17; Sigma, St. Louis), which is conserved in all mammalian species investigated (Fig. 3A). MnSOD protein levels were quantified from a standard curve generated with the horse cell line.

2.6. BER repair activities

All major steps of the BER pathway were investigated directly in cells from eight mammalian species. Specific oligonucleotides used in these assays are presented in Table 2. All activities were determined essentially as described by Stuart et al. (2004).
Briefly, APE activities were determined by the incubation of 100 ng of whole cell extract with 1 pmol of \( ^{32} \text{P} \)-end labeled THF-containing double stranded 30mer oligonucleotide (THF; Table 2) for 5, 10 and 15 min, at 37 °C in a 10 µl reaction buffer containing 50 mM HEPES-KOH (pH7.5), 50 mM KCl, 100 µg/ml BSA, 100 mM MgCl₂, 10% glycerol and 0.05% Triton X-100. Reactions were terminated by addition of 10 µl of formamide loading buffer and heated at 90 °C for 10 min. Two controls were run in parallel to APE activity assays: the addition of control oligonucleotide (no gap); no cell lysate added.

Polymerase β (β-pol) gap-filling activity was determined by the incubation of 1 µg whole cell extract with 1 pmol gapped oligonucleotide (GAP; Table 2) for 30, 60 and 90 min, at 37 °C in a 10 µl reaction buffer containing 40 mM HEPES, 0.1 mM EDTA, 5 mM MgCl₂, 0.2 mg/ml BSA, 50 mM KCl, 1 mM DTT, 40 mM phosphocreatine, 100 µg/ml phosphocreatine kinase, 2 mM ATP, 40 µM dCTP, 4 µCi \( ^{32} \text{P} \)-dCTP, 3% glycerol. Reactions were terminated by the addition of 5 µg proteinase K and 1 µl 10% SDS and incubating at 55 °C for 30 min. DNA was precipitated by addition of 1 µg glycogen, 4 µl 11 M ammonium acetate and 30 µl ethanol, overnight at −20 °C. Samples were centrifuged, dried, suspended in 10 µl formamide loading buffer (80% formamide, 10 mM EDTA, 1 mg/ml xylene cyanol FF, and 1 mg/ml bromophenol blue) and incubated at 90 °C for 10 min. Two controls were run in parallel to β-pol activity assays: the addition of control oligonucleotide (no gap); no cell lysate added.

All activity assay products were resolved by electrophoresis at 20 W for 2 h on 20% polyacrylamide gels containing 7 M urea. Bands were visualized by Phosphorimager (Fujifilm FLA-3000) and analyzed with ImageGauge software. Quantification was influenced by the half-life of \( ^{32} \text{P} \)-dCTP. A conversion was used based on the known 14d half-life of the radionuclide.

2.7. Statistical analysis

Pearson product moment correlational analysis was carried out on antioxidant and BER enzyme activities to determine if the slopes of the line of best fit were significantly different to horizontal or not. The presence of a horizontal line indicates that there is no correlation between the two variables. One-tailed paired Student’s \( t \)-tests were carried out to compare antioxidant and BER enzyme activities at the two oxygen levels.

3. Results

3.1. Fibroblast catalase and glutathione peroxidase activities do not correlate with species lifespan

Stress resistance scales positively with mammalian MLS (Kapahi et al., 1999) when cells are cultured under stdO₂. To understand the factors underlying this relationship, the correlation of antioxidant enzyme and DNA BER activities with MLS was investigated. CAT, localized in peroxisomes, reduces hydrogen peroxide to water and oxygen. Although, catalase activity shows a positive trend with species MLS in cells cultured at stdO₂ (media \( \text{PO}_{2} \sim 170 \text{ mmHg} \)), this relationship was not lost completely. On average, CAT activity was upregulated three-fold when cells are cultured under high oxygen tension. Data represent the means ± S.E. of duplicate measurements of three independently prepared cell lysates. The \( r \) values indicate Pearson product moment correlation coefficients, and the \( p \) values denote levels of statistical significance.

Table 2

<table>
<thead>
<tr>
<th>Oligonucleotide substrates used</th>
<th>Name</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>dsC</td>
<td>5'-ATA TAC CGC GGC CGG CCG ATC AAG CTT ATT-3'</td>
<td></td>
</tr>
<tr>
<td>THF</td>
<td>5'-TAT ATG GCG CCG GGC GGC TAG TTC GAA TAA-5'</td>
<td></td>
</tr>
<tr>
<td>GAP</td>
<td>5'-ATA TAC CGC GGC(AP) CCG CCG ATC AAG CTT ATT-3'</td>
<td></td>
</tr>
<tr>
<td>dsG</td>
<td>5'-TAT ATG GCG CCG GGC GGC TAG TTC GAA TAA-5'</td>
<td></td>
</tr>
</tbody>
</table>

Underlined regions represent area of interest. dsC = double-stranded control with no damage; THF = double-stranded oligonucleotide containing a tetrahydrofuran apurinic/apyrimidinic (AP) site analogue; GAP = double-stranded gapped-oligonucleotide; dsG = double-stranded control oligonucleotide without gap.

![Fig. 1](image-url) Catalase activity does not correlate with species lifespan. (A) A positive trend between CAT activity and MLS was observed in cells grown at stdO₂ (●), although this relationship was not significant (although \( p < 0.1 \)). (B) When cells were cultured under a more physiological oxygen tension (3%) (○) this relationship was lost completely. On average, CAT activity was upregulated three-fold when cells are cultured under high oxygen tension.

GPx is expressed in various subcellular compartments, including mitochondria. GPx also reduces hydrogen peroxide,
using glutathione as electron donor. At either oxygen tension, GPx did not scale with species MLS, as represented by the horizontal line (stdO2 \( r = 0.199, p > 0.05 \); physO2 \( r = 0.027, p > 0.05 \)) (Fig. 2A and B). Cells cultured at stdO2 had three-fold increase in GPx activity (Table 3).

### 3.2. Positive correlation of MnSOD with species MLS

Spectrophotometric assays of superoxide activity in whole cell extracts yielded unreliable results with high non-specific background and interfering activities. As an alternative, MnSOD protein levels were determined using an antibody specific to a region of the protein that is 100% conserved in the species investigated (Fig. 3). MnSOD protein level was a strong correlate of species lifespan whether measurements were made at stdO2 (\( r = 0.883, p < 0.01 \)) (Fig. 3C) or physO2 (\( r = 0.729, p < 0.05 \)) (Fig. 3E). However, it must be noted that our MnSOD antibody failed to detect the protein in our human fibroblasts, despite the fact that the epitope was 100% conserved in humans. For this reason, no human data point is present in Fig. 3. It is possible that human MnSOD is post-translationally modified in a way that impedes the interaction of the protein with the antibody.

Since MnSOD is confined to mitochondria, differences in mitochondrial number per cell could underlie the observed relationship. To assess this possibility, citrate synthase (CS) was utilized as a proxy for mitochondrial abundance (Holloszy et al., 1970; Williams et al., 1986; Hood et al., 1989). When MnSOD protein levels were standardized to CS activities (methods as previously described Brown et al., 2007), the correlation remained, and indeed was slightly strengthened (stdO2 \( r = 0.896, p < 0.01 \); physO2 \( r = 0.734, p < 0.05 \)) (Fig. 3D and F). Similar to activities of CAT and GPx there was a general upregulation of MnSOD protein levels when cells were grown at stdO2 (Table 4), again indicating that culture oxygen levels regulate these antioxidant enzymes.

### 3.3. Negative correlation of DNA BER with donor MLS

Many of the oxidative adducts that occur in DNA, including small base damage, abasic sites, and single strand breaks, are removed via the short-patch BER pathway. In mammals, this pathway involves the sequential coordination of four enzymes, specific DNA glycosylases, AP-endonuclease I (APE), DNA polymerase \( \beta \) (\( \beta \)-pol) and DNA ligase.

APE, involved in the second step of the BER pathway, is responsible for further processing abasic sites (produced by the removal of the damaged base by a specific DNA glycosylase)
Fig. 3. Manganese superoxide dismutase protein level correlates positively with species lifespan. (A) Alignment of available sequence data indicating 100% homology between amino acid residues 183–199 (starred underline). (B) Representative Western blot analysis of MnSOD levels in dermal fibroblasts grown at stdO2 and physO2 with loading control Memcode® stained membrane. (C) Relative MnSOD protein levels grown at stdO2 (●) positively correlates with MLS. (D) The correlation between MnSOD and MLS becomes stronger when samples are corrected for citrate synthase activity, as a proxy for mitochondrial number. (E) In dermal fibroblasts cultured at physO2 (○) oxygen MnSOD correlation with species MLS is weaker than when cells are cultured under stdO2 conditions. (F) This relationship is maintained when mitochondrial number is accounted for by standardizing to citrate synthase (CS) activity. Data are means ± S.E. of duplicate measurements of three independently prepared cell lysates, r and p values as in Fig. 1. See Section 2 for details on quantification.
which results in a strand break. APE activity was determined through incision of an oligonucleotide containing a THF abasic site analogue (THF, Table 2). No correlation between APE activity and MLS was observed when dermal fibroblasts are grown at either oxygen tension (Fig. 4B and C). Surprisingly, oxygen tension did not upregulate APE activity to the same degree as other antioxidant or repair enzymes (Table 5).

Determining the efficiency of β-pol is integral to investigating the BER pathway contribution to stress-resistance, because this enzyme catalyzes what is thought to be the rate-limiting deoxyribose phosphate lyase (dRP-lyase) step (Srivastava et al., 1998). As such, if the hypothesis that longer-lived mammals have greater BER capacities is true, a relationship should be observed at the point in which flux through the pathway is limited. The ability of β-pol to fill in an oligonucleotide containing a single nucleotide gap (GAP, Table 2) was investigated in all mammalian species under conditions that forced short-patch BER. Surprisingly, β-pol activity correlated inversely with MLS at stdO2 (r = −0.854) (Fig. 5B) but not at physO2 (r = −0.644) (Fig. 5C). The addition of 0.05 µg aphidicolin, which inhibits replicative DNA polymerases, confirmed that >95% of polymerase activity was in fact a result of β-pol (data not shown). In accordance with previous measurements, stdO2 appeared to impose a mild oxidative stress, manifesting as upregulated polβ activity in virtually all cell lines (Table 5).

4. Discussion

A large body of evidence supports the theory that mammals achieve greater longevity by enhancing cellular resistance to oxidative stress (Kirkwood et al., 2000, for review). Kapahi et al. (1999) showed that this phenotype persists in cells biopsied from mammals and cultured for multiple generations, suggesting that it is an intrinsic cellular property. Here, we used a similar comparative approach, using cell lines from mammalian species with MLS spanning between five and 122.5 years, to test the hypotheses that fibroblasts from longer-lived mammals have higher antioxidant and/or DNA repair capacities. However, of the antioxidant enzymes, only MnSOD was found to correlate positively with species MLS, BER enzyme activities showed either no correlation (APE), or correlated negatively with donor species MLS (β-pol). Thus, it appears generally not to be the cases that antioxidant and repair activities are universally higher in fibroblasts of longer-lived animals, but rather only the mitochondrial antioxidant MnSOD.

<table>
<thead>
<tr>
<th>Species</th>
<th>Relative MnSOD protein levels (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stdO2</td>
</tr>
<tr>
<td>R. norvegicus</td>
<td>2.72 ± 0.49</td>
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<td>O. cuniculus</td>
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<td>O. aries</td>
<td>5.45 ± 1.14</td>
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<td>C. familiaris</td>
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<tr>
<td>B. taurus</td>
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<td>M. fascicularis</td>
<td>5.11 ± 1.47</td>
</tr>
<tr>
<td>E. caballus</td>
<td>6.84 ± 1.36</td>
</tr>
</tbody>
</table>

Data represent the means ± S.E. of duplicate measurements on cell lysates from three independent experiments (* p < 0.05 as in Table 3).
We re-analyzed the data (not shown) after excluding the rat and human data points, which represented the low and high extremes of the data set. The rationale for this analysis was that: (1) the rat donor was a non-adult, which might influence the activities of interest (Cabellof et al., 2006), and (2) the estimate of MLS for humans is unfairly biased toward very rare observations of extreme longevity that cannot be made in other species. However, very similar results were obtained in the absence of these data points and our general conclusions regarding the positive correlation of MnSOD and negative correlation of β-pol with species MLS was not altered. The finding that MnSOD levels correlated with MLS is particularly interesting given the apparent significance of this enzyme as a regulator of animal longevity. The majority of cellular ROS is thought to originate within mitochondria, via ‘electron leak’ from respiratory complexes to molecular oxygen to form superoxide (Finkel and Holbrook, 2000, for review). Thus MnSOD, in the mitochondrial matrix, represents a first line of defense in the cellular antioxidant system. This enzyme is critical to stress resistance and survival in mice (Li et al., 1997; Lebovitz et al., 2003) alters stress resistance of cells in culture. However, there is no consistent correlation between the levels of these enzymes and animal MLS. Only when targeted ectopically to mitochondria has CAT overexpression been found to alter mammalian MLS (Schriner et al., 2005). This result suggests that mitochondria are the critical site wherein oxidant production and lifespan are integrated.

DNA BER did not show the expected positive correlation with donor species MLS. Indeed, it is interesting that APE activity showed no correlation with donor species MLS; nor was it affected by the O2 level at which the cells were propagated. While this latter result is in contrast to the known inducibility of APE1 by oxidative stress (Ramana et al., 1998), it is consistent with the data of Edwards et al. (1998), who showed that hyperoxia (100% O2) elicits a substantial but transient increase in APE1 protein level in rat tissues. At 6 h following exposure to hyperoxia, APE1 levels in rat brain tissue were more than doubled. However, by 12 h, even though the rats continued to be exposed to hyperoxia, APE1 protein levels had returned to normal. In our experiments, exposure to the higher pO2 of the stdO2 condition was chronic, with between one and several weeks for cells to adapt to this condition. Therefore, any initial induction of APE activity may have stabilized by the time our measurements were made.

The negative correlation observed between polymerase β activity and species MLS was a surprising result, which runs counter to the idea that cells of longer-lived species have enhanced DNA repair. While it seems intuitive that for genomic stability to be maintained over lifespans approaching 100 years, BER activity should be significantly enhanced; experimental support for this idea is limited. Heterozygous Ape1+/− mice expressing ~50% less APE have higher mutation frequencies (Huamani et al., 2004), and are more susceptible to oxidative stress, but MLS is unaffected (Meira et al., 2001). Similarly, heterozygous β-pol+/− mice have ~50% less β-pol protein and CS activity, or standardization of total SOD to metabolic rate, did not abolish the correlation. Thus, both results suggest a correlation between SOD and lifespan in mammals.

In contrast to MnSOD, no evidence was found for a correlation of CAT or GPx activities with donor species MLS. Various authors have demonstrated that engineered over- or underexpression of CAT (Chen et al., 2004) or GPx (de Haan et al., 2003) alters stress resistance of cells in culture. However, there is no consistent correlation between the levels of these enzymes and animal MLS. Only when targeted ectopically to mitochondria has CAT overexpression been found to alter mammalian MLS (Schriner et al., 2005). This result suggests that mitochondria are the critical site wherein oxidant production and lifespan are integrated.

Table 5
<table>
<thead>
<tr>
<th>Species</th>
<th>APE stdO2</th>
<th>APE physO2</th>
<th>Polymerase β stdO2</th>
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<td>R. norvegicus</td>
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<td>0.0408</td>
<td>28.9 ± 1.66</td>
<td>18.6 ± 5.27</td>
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<tr>
<td>O. cuniculus</td>
<td>0.0339</td>
<td>0.0846</td>
<td>32.2 ± 0.887</td>
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<td>O. aries</td>
<td>0.0677</td>
<td>0.0707</td>
<td>29.3 ± 2.12</td>
<td>18.0 ± 4.68</td>
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<td>C. familiaris</td>
<td>0.0542</td>
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<td>21.5 ± 2.62</td>
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<td>H. sapiens</td>
<td>0.103</td>
<td>0.0317</td>
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<td>8.07 ± 1.46</td>
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</table>

APE = AP endonuclease activity (units = pmo/min/mg); polymerase β activity units = Δsignal strength/min/μg. Data represent the means ± S.E. of duplicate measurements on cell lysates from three independent experiments (p < 0.05 as in Table 3).
activity in various tissues (Cabelof et al., 2003a) but both wild-type and β-pol\textsuperscript{+/−} mice have similar mean and maximum lifespans (Cabelof et al., 2006). This suggests that β-pol activity in vivo also may not correlate positively with mammalian lifespan.

β-pol expression and overall BER capacity is upregulated in vivo by oxidative stress (Cabelof et al., 2002), and mitochondria of longer-lived species generate less ROS (e.g. Ku et al., 1993; Lambert et al., 2007). We therefore investigated whether β-pol activity in fibroblasts in vitro might be related to inter-species

Fig. 5. Polymerase β activity inversely correlates with species lifespan. (A) β-pol activity was determined by measuring the activity of β-pol to fill in a single nucleotide gap oligonucleotide at 30, 60 and 90 min, standardized to 1 μg whole cell lysate. The 16-mer corresponds to the product of β-pol \textsuperscript{32}P-dCTP incorporation and the 34-mer corresponds to the fully repaired and ligated product, representing both β-pol and DNA ligase activities. For analysis of β-pol activity, the kinetics of the appearance of 16-mer was quantified. Control = control oligonucleotide (no gap); no protein = no cell lysate added; pure human β-pol and T4 DNA ligase were used as a positive control demonstrating \textsuperscript{32}P-dCTP incorporation and subsequent ligation. (B) Under \textsubscript{std}O\textsubscript{2} (●) conditions β-pol negatively correlates with MLS. (C) Conversely, β-pol does not correlate with MLS when fibroblasts are grown under \textsubscript{phy}O\textsubscript{2} (○) conditions. β-pol activity is upregulated when cells are cultured under \textsubscript{phy}O\textsubscript{2} conditions. Data are means ± S.E. of duplicate measurements of three independently prepared cell lysates. \textit{r} and \textit{p} values as in Fig. 1. See Section 2 for details on quantification.
differences in cellular oxidative stress. CS activity (a proxy of cellular oxidative metabolism) was a negative correlate of MLS at physO2 (Fig. 6B), and plotting β-pol activity against CS activity revealed a positive correlation (Fig. 6D). This suggests that β-pol activity may be proportional to fibroblast metabolic rate and resultant ROS production, thus indicating an adaptive response of β-pol activity to oxidative stress. Higher rates of oxidative metabolism may be associated with an increased incidence of oxidative DNA damage requiring increased repair. Increased incidence of oxidative DNA damage can also be caused simply by exposing fibroblasts to higher O2 (Parrinello et al., 2003), and we found β-pol activities were typically higher in cells grown at stdO2 (though this was not consistently statistically significant). Thus at stdO2 cells of all species probably experienced greater oxidative stress and DNA oxidative damage (Hamilton et al., 2001). Therefore, interventions that ameliorate the age-related loss of β-pol inducibility by ROS (Cabellof et al., 2003b) may be important in delaying the aging phenotype.

Taken together, our results suggest that the use of ‘normal’ cell culture conditions, i.e. those that do not regulate O2 to produce physiologically normoxic media pO2, may be inappropriate for studies in which effects of an exogenous oxidative stressor are quantified. For example, failure of cells in culture to upregulate a particular antioxidant or BER activity may simply reflect the fact that substantial induction has already occurred due to the effects of atmospheric O2. For this reason, it is probably advisable for investigators studying oxidative stress using cell culture models to regulate O2 at near-physiological levels such that the correct baseline is established prior to the experimental induction of oxidative stress.

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References


