Asymmetric Inheritance of Oxidatively Damaged Proteins During Cytokinesis
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A small, yet significant, fraction of the carrier was found in association with the TIM22 complex even in the complete absence of a membrane potential (addition of valinomycin) (Fig. 4B). To exclude a post-lysis association of the carrier with the TIM22 complex, we performed a mixing experiment. 35S-labeled carrier was imported into either wild-type mitochondria or mitochondria carrying ProtA-tagged Tim18. After the import reaction, the mitochondria were mixed and lysed with digitonin (Fig. 4D). The purified TIM22 complex carried the stage IV–intermediate only when the radiolabeled precursor was imported into Tim18, ProtA, mitochondria (Fig. 4D), which demonstrates that the association of carrier with the TIM22 complex had occurred in intact mitochondria. We asked if the fraction of carrier associated with the TIM22 complex in the absence of a membrane potential (“tethered form”) was bound in a different way to the stage IV intermediate at low membrane potential (“docked form”). Perhaps in the absence of a $\Delta \psi$, the carrier preferentially associates with the small Tim proteins of the TIM22 complex that interact with the hydrophobic segments of carrier proteins (19), whereas in the presence of a low $\Delta \psi$, the carrier comes into contact with the pore where ion interactions could also take place. The carrier can contact the small Tim proteins at the TIM22 complex (including Tim12) in the absence of a $\Delta \psi$ (15, 20), whereas contact to Tim22 requires the membrane potential (21). Indeed, when the carrier was arrested at the different steps the tethered precursor was significantly more resistant to increasing ionic strength of the buffer than the docked precursor (Fig. 4E). Thus, tethered and docked forms of the carrier reside in different molecular environments at the TIM22 complex, the tethered protein probably being in a more hydrophilic environment.

We conclude that the interaction of carrier proteins with the TIM22 complex occurred in three steps. (i) Tethering of the precursor to the translocase occurred in a $\Delta \psi$-independent manner. (ii) The first of two voltage-dependent steps resulted in docking of the precursor in the translocase. A $\Delta \psi$ below 60 mV, which did not influence the activity of the channel itself, was sufficient for the docking step, indicating that $\Delta \psi$ was acting on the precursor protein. Because the matrix-exposed loops of the carrier proteins have a net positive charge (22, 23), an electrophoretic effect of the $\Delta \psi$ on the translocation of the loops is conceivable (18, 24, 25). (iii) In the presence of a carrier signal peptide, a membrane potential above 70 mV then activated channel closing of one channel pore, possibly to keep the inserted transmembrane segments tightly in the translocase, and the concomitant rapid gating activity of the other channel pore to promote the insertion of additional transmembrane segments. Finally, the carrier protein is laterally released into the lipid phase of the inner membrane. The single energy source, $\Delta \psi$, thus plays a dual role in protein insertion by acting on both the precursor protein and the channel.

References and Notes
6. Materials and methods are available as supporting material on Science Online.

Asymmetric Inheritance of Oxidatively Damaged Proteins During Cytokinesis
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Carbonylated proteins were visualized in single cells of budding yeast Saccharomyces cerevisiae, revealing that they accumulate with replicative age. Furthermore, carbonylated proteins were not inherited by daughter cells during cytokinesis. Mother cells of a yeast strain lacking the sir2 gene, a Sir2p-dependent sirtuin, failed to retain oxidatively damaged proteins during cytokinesis. These findings suggest that a genetically determined, Sir2p-dependent asymmetric inheritance of oxidatively damaged proteins may contribute to free-radical defense and the fitness of newborn cells.

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R E P O R T S
increases with the yeast replicative age, we isolated cells of different age from a population growing exponentially in YPD medium by elutriation. Newly born cells are small and pass through the G1 phase until they reach the critical size required to enter S phase. Thus, elutriation fractions 1 and 2 contain cells of similar replicative age, but the cells of fraction 2 are chronologically older (Fig. 1) and a small portion of cells is budding in this fraction. Fraction 2 cells exhibited a fourfold higher level of oxidative damage than fraction 1 cells (Fig. 1), implying that oxidation of proteins occurs during the first G1 phase of newborn cells. Protein oxidation increased further as mother cells produce 10 or more daughters (Fig. 1, fraction 5). The results were confirmed with the use of biotin-streptavidin magnetic sorting (8) to isolate cells of different replicative age (9). To address how and when daughter cells rid themselves of damaged proteins, we visualized carbonylated proteins in situ using immunocytochemistry (9, 10). An uneven distribution of oxidized proteins between mother cells and buds was observed during cytokinesis (Fig. 2, A and B). Paraquat exposure increased the oxidative damage (per unit volume) in the bud (Fig. 2C), demonstrating that target carbonyl groups can be effectively derivatized in the bud compartment and reached by the antibodies. Analysis of carbonylation per total protein by dot-blot analysis confirmed a lower (3.6-fold) damage density in newborn daughters. In addition, planes separated by 0.1 μm in depth (Z-series) were scanned to construct three-dimensional images of carbonylated proteins, which confirmed an unequal density of oxidatively damaged proteins in the bud and mother cells (Fig. 2D). Buds contained active mitochondria (Fig. 2E), and in situ detection of superoxide (Fig. 2F) (11) and hydrogen peroxide (9) demonstrated that the production of reactive oxygen species (ROS) was not different in mothers and buds. The carbonyl signals partly colocalized with mitochondria, but carbonyls were also detected in areas free of mitochondria (Fig. 2G).
In accordance with this, isolated mitochondria contained about 40% of total protein carbonyls in cell extracts (fig. S1).

To determine whether oxidized proteins were inherited asymmetrically during cytokinesis, we treated cells with paraquat to increase the level of carbonylated proteins two-fold, and then the mother cells were allowed to produce offspring. After elutriation, carbonylation damage per total protein in the first-generation daughters was sixfold lower than in the mother cell (Table 1). Thus, the extra load of oxidative damage appeared to be retained in the mother cell and was not inherited by the daughter cell. Consistent with this notion, the rate of degradation of carbonylated proteins was similar in mother cell and bud compartments (fig. S2).

The ability of mother cells to retain oxidatively damaged proteins during cell division diminished with replicative age (Fig. 3A). In mutant cells lacking the oxidative stress defense genes sod1, sod2, or etal (12, 13), the overall protein oxidation was higher than in wild-type cells, but the low oxidation density in buds as compared with mothers was not affected (Fig. 3B). When mutants (sir2, sir3, and sir4) displaying a reduced life-span (+) were analyzed, only cells lacking sir2 differed from the wild type (Fig. 3B). The sir2 mutants tend to simultaneously express the α and α mating-types genes (14). However, this phenotype was not linked to segregation defects because Δsir3 and Δsir4 mutants share this mating-type defect with the Δsir2 mutant (+). Deleting hml in a Δsir2 mutant did not correct for its failure to segregate carbonylated proteins (Fig. 3B). Young Δsir2 cells exhibited higher levels of oxidatively damaged proteins than young wild-type cells, and protein carbonyls were distributed evenly between mother and daughter cells during cell division regardless of the age of the Δsir2 mother cells (Fig. 3C). No difference in paraquat sensitivity, ROS production, or the state of respiration (+) was observed between the wild type and the Δsir2 mutant (fig. S3). This is consistent with a recent report demonstrating that the Δsir2 mutation does not affect the cells’ ability to withstand external oxidative stress (15). In contrast to wild-type daughters, the first-generation daughters of sir2 cells inherited the extra load of oxidatively damaged proteins after paraquat treatment (Table 1). This indicates that Δsir2 cells are defective in the segregation of oxidized proteins during cytokinesis.

The spatial distribution of F-actin during cytokinesis was atypical in the Δsir2 mutant but not in the Δsir3 and Δsir4 (fig. S4). Specifically, at the end of cytokinesis, actin was predominantly found in the daughter cells of Δsir2 mutants, whereas in wild-type, Δsir3, and Δsir4 cells, the actin is redistributed to both the mother and daughter cell (fig. S4). Inhibition of actin assembly by latrunculin A (Lat-A) abolished the ability of wild-type mother cells to retain oxidized proteins (fig. S4), suggesting that the actin skeleton is required for proper segregation of oxidized proteins.

In summary, oxidatively damaged proteins are inherited asymmetrically during yeast cytokinesis, and this process is Sir2p dependent. Although the data provided do not establish the capacity to segregate oxidative damage as a life-span determinant, they indicate a new aspect of cell division asymmetry and a mechanism for dealing with oxidative damage, which is likely important for the fitness of newly born cells.

**References and Notes**

9. Materials and Methods are available as supporting material on Science Online.
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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/1080418/DC1

Materials and Methods

References

Figs. 51 to 54

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**Table 1.** Density of oxidative damage (carbonyls per total protein) in newborn daughters as compared with the mother determined by dot-blot analysis. The “daughter fraction” is fraction 1 and the “mother fraction” is fractions 2, 3, 4, and 5 combined (fig. 1). The analysis was performed in growing untreated cultures (−) and immediately after exposure to paraquat (400 μg/ml) for 20 min (+). After paraquat treatment, the cells were washed and allowed to divide once in YPD. Mothers and newborn daughters were then isolated by elutriation and scored for carbonyl levels (column “−→+”). The oxidation density in the daughters is related to that of the mother cell, which was assigned a value of 100. Numbers in parentheses are standard deviations.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Paraquat treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt</td>
<td>28 (5)</td>
</tr>
<tr>
<td>Δsir2</td>
<td>90 (9)</td>
</tr>
</tbody>
</table>

Molecular genetics has played an important role in cancer risk assessment for rare hereditary cancer syndromes, such as CRC in familial adenomatous polyposis coli and hereditary nonpolyposis CRC (1). However, these syndromes cumulatively account for <1% of

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**Loss of IGF2 Imprinting: A Potential Marker of Colorectal Cancer Risk**

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Loss of imprinting (LOI), an epigenetic alteration affecting the insulin-like growth factor II gene (IGF2), is found in normal colonic mucosa of about 30% of colorectal cancer (CRC) patients, but it is found in only 10% of healthy individuals. In a pilot study to investigate the utility of LOI as a marker of CRC risk, we evaluated 172 patients at a colonoscopy clinic. The adjusted odds ratio for LOI in lymphocytes was 5.15 for patients with a positive family history [95% confidence interval (95% CI), 1.70 to 16.96; probability P = 0.002], 3.46 for patients with adenomas (95% CI, 1.14 to 11.37; P = 0.026), and 21.7 for patients with CRC (95% CI, 3.48 to 153.6; P = 0.0005). LOI can be assayed with a DNA-based blood test, and it may be a valuable predictive marker of an individual’s risk for CRC.