Interestingly, oxytocin administration only increased mutual gaze duration in female dogs, whereas sex differences were not observed in experiment 1, which did not include unfamiliar individuals. Sex differences in the effects of intranasal oxytocin have been reported in humans as well (*22*), and it is possible that females are more sensitive to the affiliative effects of oxytocin or that exogenous oxytocin may also be activating the vasopressin receptor system preferentially in males. Oxytocin and the structurally related vasopressin affect social bonding and aggression in sexually dimorphic manners in monogamous voles (*8*, *9*), and oxytocin possibly increases aggression (*23*, *24*). Therefore, the results of experiment 2 may indicate that male dogs were attending to both their owners and to unfamiliar people as a form of vigilance. The current study, despite its small sample size, implies a complicated role for oxytocin in social roles and contexts in dogs.

In human infants, mutual gaze represents healthy attachment behavior (*25*). Human functional magnetic resonance imaging studies show that the presentation of human and canine family members' faces activated the anterior cingulate cortex, a region strongly acted upon by oxytocin systems (*26*). Urinary oxytocin variation in dog owners is highly correlated with the frequency of behavioral exchanges initiated by the dogs' gaze (*19*). These results suggest that humans may feel affection for their companion dogs similar to that felt toward human family members and that dog-associated visual stimuli, such as eye-gaze contact, from their dogs activate oxytocin systems. Thus, during dog domestication, neural systems implementing gaze communications evolved that activate the humans' oxytocin attachment system, as did gaze-mediated oxytocin release, resulting in an interspecies oxytocin-mediated positive loop to facilitate human-dog bonding. This system is not present in the closest living relative of the domesticated dog.

In the present study, urinary oxytocin concentrations in owners and dogs were affected by the dog's gaze and the duration of dog-touching. In contrast, mutual gaze between hand-raised wolves and their owners was not detected, nor was there an increase of urinary oxytocin in either wolves or their owners after a 30-min experimental interaction (experiment 1). Moreover, the nasal administration of oxytocin increased the total amount of time that female dogs gazed at their owners and, in turn, urinary oxytocin concentrations in owners (experiment 2). We examined the association between our results and early-life experience with humans in dogs and wolves in order to test the possibility that our results were due to differences in early-life experience with humans. The results did not indicate a significant association between the animals' early-life experiences with humans and the findings of the current study (see the supplementary methods). Moreover, there were no significant differences between dogs in the long-gaze group and wolves in either the duration of dog/wolf-touching and dog/wolf-talking, suggesting that the shorter gaze of the wolves was not due to an unstable relationship. These results support the existence of a self-perpetuating oxytocin-mediated positive loop in human-dog relationships that is similar to that of human mother-infant relations. Human-dog interaction by dogs' human-like gazing behavior brought on social rewarding effects due to oxytocin release in both humans and dogs and followed the deepening of mutual relationships, which led to interspecies bonding.

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SUPPLEMENTARY MATERIALS

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PLANT ECOLOGY

Anthropogenic environmental changes affect ecosystem stability via biodiversity

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Human-driven environmental changes may simultaneously affect the biodiversity, productivity, and stability of Earth's ecosystems, but there is no consensus on the causal relationships linking these variables. Data from 12 multiyear experiments that manipulate important anthropogenic drivers, including plant diversity, nitrogen, carbon dioxide, fire, herbivory, and water, show that each driver influences ecosystem productivity. However, the stability of ecosystem productivity is only changed by those drivers that alter biodiversity, with a given decrease in plant species numbers leading to a quantitatively similar decrease in ecosystem stability regardless of which driver caused the biodiversity loss. These results suggest that changes in biodiversity caused by drivers of environmental change may be a major factor determining how global environmental changes affect ecosystem stability.

Human domination of Earth's ecosystems, especially conversion of about half of the Earth's ice-free terrestrial ecosystems into cropland and pasture, is simplifying ecosystems via the local loss of biodiversity (*1*, *2*). Other major global anthropogenic changes include nutrient eutrophication, fire suppression and elevated fire frequencies, predator decimation, climate warming, and drought, which likely affect many aspects of ecosystem functioning, especially ecosystem productivity, stability, and biodiversity (*1*, *3*–*7*). However, to date there has been little evidence showing whether or how these three ecosystem responses may be mechanistically

linked. Rather, at present each anthropogenic driver of environmental change has been considered to have its own idiosyncratic syndrome of impacts on ecosystem productivity, stability, and biodiversity (*1*, *5*–*10*).

This perspective was recently called into question by a study showing that the initial impacts of nutrient addition on grassland productivity were reduced through time in proportion to the extent to which nutrient addition led to the loss of plant diversity (*11*). In essence, that study suggested that the positive dependence of productivity on plant diversity (*12*–*17*), in combination with the negative effect of eutrophication on diversity (*8*, *18*), caused the initial increase in productivity with nitrogen enrichment to diminish over time due to the loss of plant diversity caused by chronic nitrogen fertilization (*11*). This suggests the hypothesis that other drivers of global environmental change may have biodiversity-mediated effects on ecosystem functioning (*19*)—that changes in biodiversity resulting from anthropogenic drivers may be an intermediate cause of subsequent changes in ecosystem functioning. Here we test this hypothesis. Numerous biodiversity experiments have shown that reduced plant diversity leads to decreased temporal stability of productivity because of reductions in compensatory dynamics or in asynchronous responses to environmental fluctuations (*12*, *16*, *20*, *21*). Here, our test determines how experimental manipulations of nitrogen (N), carbon dioxide (CO_2), fire, herbivory, and water affect biodiversity and productivity; and if changes in ecosystem stability associated with each environmental driver have the same dependence on biodiversity as observed in biodiversity experiments, or if each driver has an individualistic impact on stability (*5*, *6*).

We perform this particular test because, whereas effects of anthropogenic drivers on biodiversity and productivity have been widely investigated (*5*, *6*, *11*), their long-term impacts on the temporal stability of productivity have received less attention, and the few published studies examining a single driver report mixed results (*7*, *9*, *10*, *22*–*25*). A commonly used measure of stability among many proposed in the ecological literature (*26*, *27*) defines the temporal stability of productivity (S) as the ratio of the temporal mean of productivity to its temporal variability as measured by its standard deviation (SD) (*28*). This measure of stability is the inverse of the coefficient of variation. Under this definition, a driver could increase stability by increasing the mean productivity relative to the SD, by decreasing the SD relative to the mean productivity, or both. Drivers that increase the SD may also increase stability if there is a correspondingly larger proportional increase in mean productivity (or vice versa) (*7*, *20*, *29*). Importantly, given that the temporal mean and SD of productivity can depend on biodiversity (*7*, *21*, *29*), drivers might influence stability through their long-term effects on biodiversity. The simultaneous impacts of various drivers on ecosystem biodiversity, productivity, and stability have not previously been explored, thus limiting our current understanding.

Here, we determine if ecologically or societally relevant magnitudes of change in six important anthropogenic drivers influence the stability of ecosystem productivity and whether changes in stability correspond with changes in biodiversity. In particular, we test the hypothesis that changes in biodiversity, regardless of the causal factor, consistently affect the stability of ecosystem productivity.

We used data from 12 experiments that manipulated one or more anthropogenic drivers over a period of 4 to 28 years (table S1). We examine both long-term stability (temporal stability determined using all 4 to 28 years of data collected on aboveground biomass in each experiment) and short-term stability (the temporal stability of each 3-year period of each experiment) and the dependence of these metrics of stability on the concurrent measures of plant species numbers.

We begin by evaluating the extent to which changes in grassland plant diversity, whether experimentally manipulated or in response to other anthropogenic drivers, including N, CO_2, fire, herbivory, and water, predict changes in the long-term temporal stability of productivity. Our analyses control for what otherwise might be potentially confounding variables by including only experiments at the Cedar Creek Ecosystem Science Reserve on well-drained sandy soils of east-central Minnesota, USA, that used perennial grassland ecosystems of similar plant species compositions (*5*). We determined long-term temporal stability, S, as μ/σ, where μ is the average productivity of a plot across all years and σ is the temporal standard deviation in the productivity of that plot across all years. We calculated long-term stability responses as the natural logarithm of the ratio (log response ratio or lrr) of the long-term stability within each treatment plot divided by the average long-term stability in the reference plots (lrr.S). Similarly, we calculated the associated plant species richness responses as the natural logarithm of the ratio of the average richness across all years within each treatment plot divided by the average richness across all years in the reference plots (lrr.rich). Log response ratios quantify the proportional change in treatment plots relative to reference plots. Because lrr.S is the difference between the log response ratio of the temporal mean (lrr.mean) and the log response ratio of the temporal standard deviation (lrr.SD), it separates the effects of anthropogenic drivers on stability into their simultaneous effects on the mean and variance of productivity.

Reference plots were unmanipulated or otherwise had more historically typical conditions, such

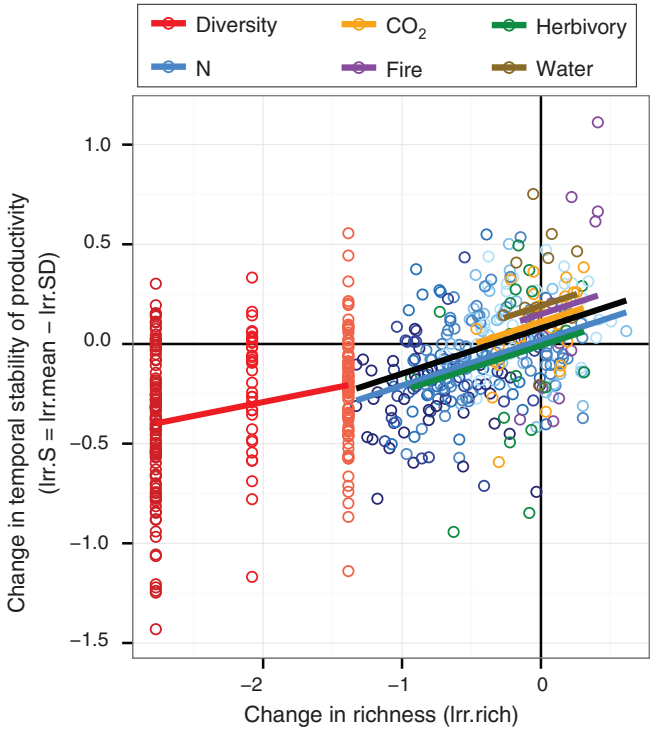

Fig. 1. Human-driven environmental changes affect ecosystem stability via biodiversity. Effect of anthropogenic drivers of environmental change on the stability of productivity, as mediated by experimentally imposed changes in biodiversity [red line; slope and 95% confidence intervals (CIs): 0.14 (0.08 to 0.20)], or from biodiversity changes arising from anthropogenic drivers including N, CO_2, water, fire, and herbivory [black and other colored lines; slopes and 95% CIs: 0.22 (0.15 to 0.31)]. Black and red lines are based on separate fits; their similar slopes show that changes in biodiversity caused by anthropogenic drivers have effects on stability similar to those resulting from experimentally imposed changes in plant biodiversity ($F_{1,561}$ = 3.29, P = 0.07). Relative changes were calculated as the natural logarithm of the ratio (lrr) of the variable within each treatment plot divided by the average of the variable in the reference plots. Black line is the fixed-effect linear regression slope across all anthropogenic drivers in the mixed-effects model; colored lines show trends for each driver. Colors for the points correspond to treatments in Fig. 2.

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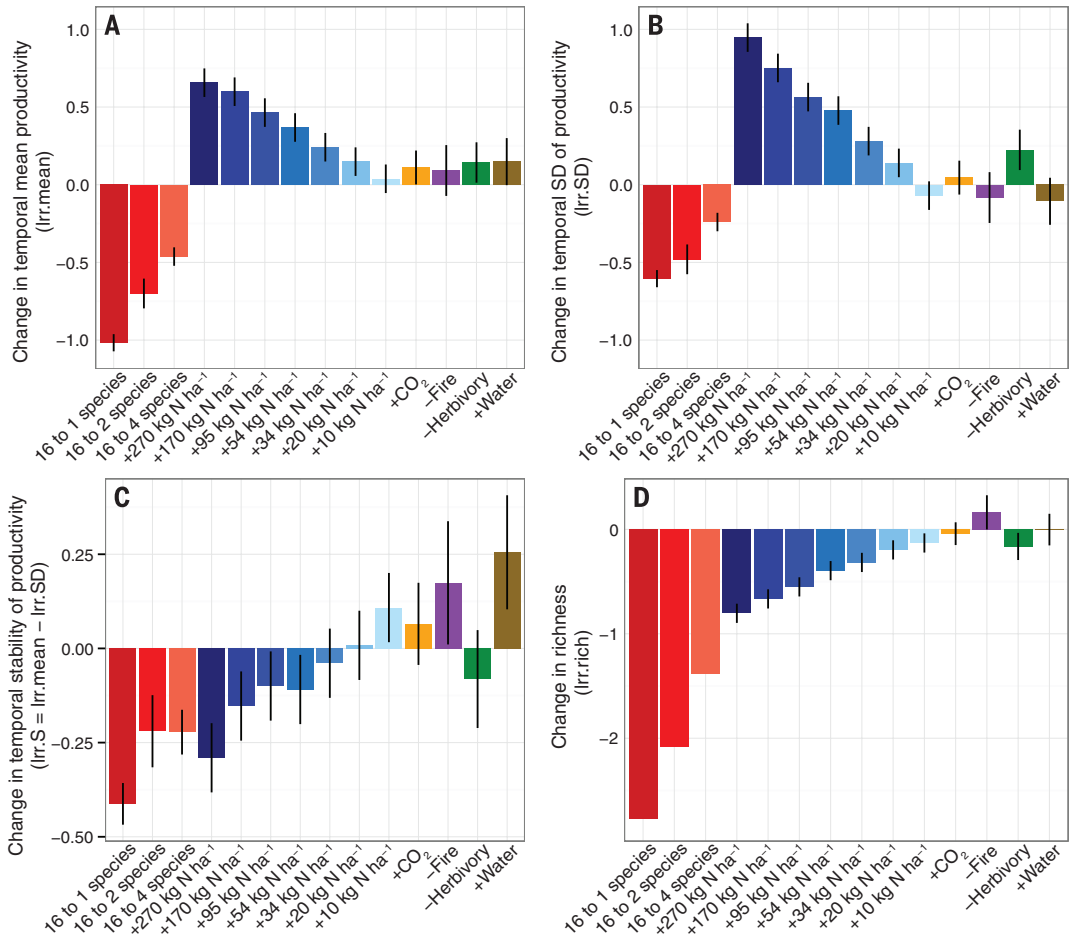

Fig. 2. Simultaneous effect of human-driven environmental changes on ecosystem productivity, stability, and biodiversity. Effect of anthropogenic drivers of environmental change on relative changes in the (**A**) mean, (**B**) standard deviation (SD), (**C**) stability of ecosystem productivity, and (**D**) plant diversity. Treatment effects are shown with their 95% CI such that treatments with intervals overlapping zero are not significantly different from zero (table S4).

as high diversity or ambient N, CO_2, herbivory, and water conditions or presettlement fire conditions. In particular, we compared biodiversity from plots planted with one, two, and four species to reference plots planted with 16 species, a level representative of a high-diversity (16.3 species m^{-2}) natural grassland community in this area (*5*). N additions of 270, 170, 95, 54, 34, 20, and 10 kg ha^{-1} were compared to plots receiving no N, and addition of CO_2 and water, fire suppression, and herbivore exclusion were compared to grassland plots with ambient or presettlement conditions. These treatments (except 270, 170, and 95 kg N ha^{-1} and perhaps the monocultures of biodiversity experiments) also fall within the ranges occurring in natural grassland ecosystems of this region (*5*).

We found that changes in plant diversity in response to anthropogenic drivers, including N, CO_2, fire, herbivory, and water, were positively associated with changes in temporal stability of productivity (black line in Fig. 1; Fig. 2, C and D). This positive association was independent of the nature of the driver, resulting in parallel relationships (all colored lines except red in Fig. 1; table S2). This suggests that biodiversity-mediated effects on stability are independent of the factor driving changes in biodiversity. Moreover, the positive association between changes in plant diversity and changes in stability in response to anthropogenic drivers was similar to that observed in two neighboring experiments that directly manipulated plant diversity (compare the black and red lines in Fig. 1) (*21*). Thus, changes in biodiversity resulting from anthropogenic environmental changes have similar effects on stability as observed in biodiversity experiments, suggesting that changes in biodiversity may be an intermediary factor influencing how anthropogenic environmental changes affect ecosystem stability. For example, whether a 30% change in plant diversity (lrr.rich = −0.357) resulted from elevated N, CO_2, or water or from herbivore exclusion, fire suppression, or direct manipulation of plant diversity, stability tended to decrease in parallel by 8% (lrr.S = −0.082). This conclusion is supported by analyses showing that there was no remaining effect of anthropogenic drivers on changes in stability after biodiversity-mediated effects were taken into account (table S3) and that changes in stability based on biodiversity manipulations predict changes in stability in response to anthropogenic drivers (fig. S1).

We next evaluated the extent to which changes in temporal stability of productivity in response to anthropogenic drivers were caused by changing the temporal mean of productivity or the temporal variance of productivity. We found that when a driver of environmental change caused mean productivity to change, it did not consistently lead to higher or lower stability of productivity (Fig. 2 and table S4). For example, decreases in biodiversity from 16 species to one, two, and four species decreased both the temporal mean and stability of productivity (Fig. 2, A and C). By contrast, addition of N, CO_2, and water; fire suppression; and herbivore exclusion generally increased the temporal mean of productivity, although not always significantly (Fig. 2A), but either increased (N addition of 10 kg ha^{-1}, fire suppression, and water addition), reduced (N addition of 270, 170, 95, and 54 kg ha^{-1}), or had no detectable effects (N addition of 34 and 20 kg ha^{-1}, addition of CO_2, and herbivore exclusion) on stability (Fig. 2C). These differing effects on stability (Fig. 2C) were due to differences in the direction and magnitude of drivers' impact on mean productivity (Fig. 2A) compared to their variance (Fig. 2B). For example, experimental decreases in biodiversity caused a larger decrease in mean productivity than in its variance, resulting in decreased stability; whereas N addition of 10 kg ha^{-1}, fire suppression, and water addition each caused a larger increase in mean productivity

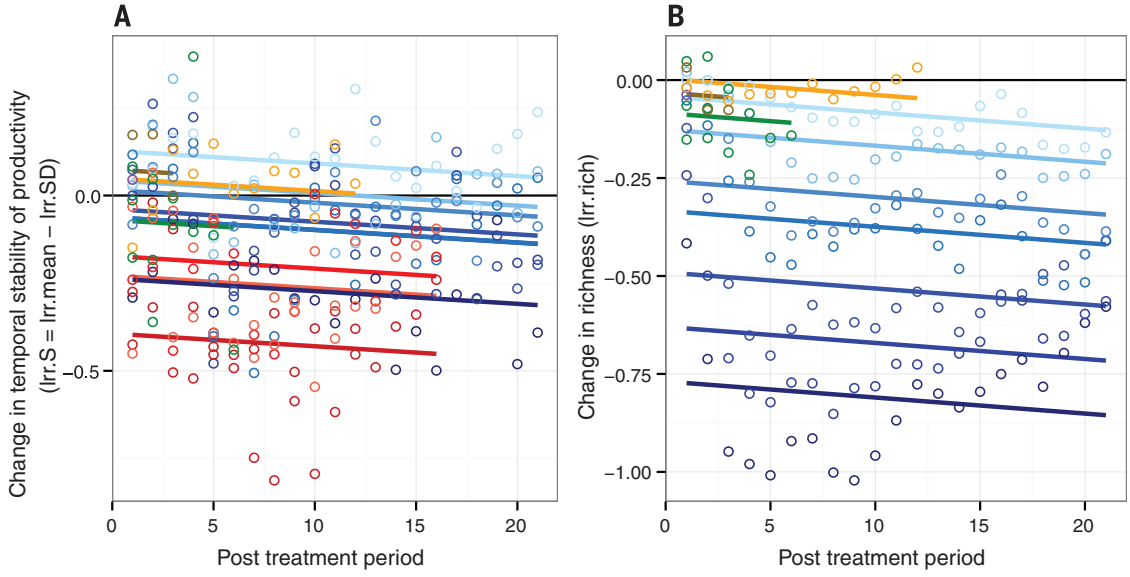

Fig. 3. Temporal trends in effect sizes of ecosystem stability and biodiversity responses to anthropogenic drivers of environmental change. Effects of anthropogenic drivers on (**A**) stability ($F_{13,220}$ = 30.6, P < 0.001) and (**B**) diversity ($F_{10,154}$ = 103.3, P < 0.001) were consistent through time (Drivers × Time: P > 0.1 in both cases). Stability ($F_{1,154}$ = 86.5, P < 0.001) and diversity ($F_{1,220}$ = 24.8, P < 0.001) had a weak tendency to decrease with increasing treatment duration. Data were divided into overlapping intervals of 3 years reported as posttreatment period after initiation of the experiment (*31*), with diversity and stability determined for each interval. Colors for the points and lines correspond to treatments in Fig. 2.

than in its variance, resulting in increased stability. By contrast, N addition of 270, 170, 95, and 54 kg ha^{-1} caused a larger increase in the variance than the mean, resulting in reduced stability. We do not expect the direction and magnitude of changes in the numerator or denominator of the stability ratio to be universal. For example, in other biodiversity experiments, decreases in biodiversity caused a larger decrease in the variance of productivity than the mean (*29*). Our results, however, do indicate that drivers consistently reduce stability when they reduce biodiversity.

Together, these results suggest that changes in biodiversity, whether experimentally manipulated or in response to other anthropogenic drivers, caused consistent changes in ecosystem stability of productivity (Figs. 1 and 2, C and D) not because of consistent effects of a driver or biodiversity on either the temporal mean of productivity or on its temporal variance (Fig. 2, A and B) but rather because of consistent effects on their ratio, which is stability (Figs. 1 and 2, C and D). The repeatedly observed quantitative effects of changes in biodiversity on ecosystem stability in this study are consistent with predictions of ecosystem stability by models of interactions among species that coexist because of interspecific trade-offs (*30*). They are also consistent with results of numerous biodiversity experiments (*29*).

We found no evidence that biodiversity-mediated effects on stability were caused by similar shifts in the abundances of functional groups or species (fig. S2). For example, although diversity and stability declined, native perennial C_4 grasses increased under herbivory exclusion (e.g., *Sorghastrum nutans*) and declined under high levels of chronic nitrogen enrichment (e.g., *Schizachyrium scoparium*), while non-native perennial C_3 grasses declined under herbivory exclusion (e.g., *Koeleria cristata*) and increased under high levels of chronic nitrogen enrichment (e.g., *Agropyron repens*). Thus, various drivers led to similar changes in stability by causing changes in biodiversity, even though the various drivers had different effects on functional groups and particular species.

We also assessed whether the diversity and stability responses were consistent through time by dividing the 4 to 28 years of annual data into overlapping intervals of three consecutive years and calculating short-term stability and average species richness for each interval. This allows us to account for the effects of the different duration of the experiments (*31*). Effects of anthropogenic drivers on diversity and short-term stability were consistent through time. Specifically, diversity and stability had a weak tendency to decrease in unison with increasing treatment duration independently of the nature of the driver, resulting in parallel negative relationships (Fig. 3). These results further suggest that the decrease in stability over time was associated with declining plant diversity in response to anthropogenic drivers.

In total, we found that the loss of plant diversity was associated with decreased stability not only in experiments that manipulate diversity (*20*, *21*) but also when biodiversity changed in response to other anthropogenic drivers. In combination with recent demonstrations that biodiversity is a major determinant of productivity (*5*, *6*, *11*), these findings suggest that any drivers of environmental change that affect biodiversity are likely to have long-term ecosystem impacts that result from these changes in biodiversity (*19*). Furthermore, biodiversity-mediated effects on stability did not qualitatively depend either on the particular factor that caused the change in biodiversity or on shifts in the abundance of particular functional groups or species. Altogether, our multiyear experiments suggest that there may be a universal impact of biodiversity change on ecosystem stability in response to anthropogenic environmental changes, with decreased plant species numbers leading to lower ecosystem stability regardless of the cause of biodiversity loss. Our work suggests that conservation policies should encourage management procedures that restore or maintain natural levels of biodiversity or minimize the negative impacts of anthropogenic global environmental changes on biodiversity loss to ensure the stable provision of ecosystem services.

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31. Materials and methods are available as supplementary materials on *Science* Online.

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SUPPLEMENTARY MATERIALS

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STEM CELLS

Asymmetric apportioning of aged mitochondria between daughter cells is required for stemness

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By dividing asymmetrically, stem cells can generate two daughter cells with distinct fates. However, evidence is limited in mammalian systems for the selective apportioning of subcellular contents between daughters. We followed the fates of old and young organelles during the division of human mammary stemlike cells and found that such cells apportion aged mitochondria asymmetrically between daughter cells. Daughter cells that received fewer old mitochondria maintained stem cell traits. Inhibition of mitochondrial fission disrupted both the age-dependent subcellular localization and segregation of mitochondria and caused loss of stem cell properties in the progeny cells. Hence, mechanisms exist for mammalian stemlike cells to asymmetrically sort aged and young mitochondria, and these are important for maintaining stemness properties.

Stem cells can divide asymmetrically to generate a new stem cell and a progenitor cell that gives rise to the differentiated cells of a tissue. During organismal aging, it is likely that stem cells sustain cumulative damage, which may lead to stem cell exhaustion and eventually compromise tissue function (*1*). To slow the accumulation of such damage, stem cells might segregate damaged subcellular components away from the daughter cell destined to become a new stem cell. Although nonmammalian organisms can apportion certain non-nuclear cellular compartments (*2*–*4*) and oxidatively damaged proteins (*5*, *6*) asymmetrically during cell division, it is unclear whether mammalian stem cells can do so as well (*6*–*9*).

We used stemlike cells (SLCs) recently identified in cultures of immortalized human mammary epithelial cells (*10*) to investigate whether mammalian stem cells can differentially apportion aged, potentially damaged, subcellular components, such as organelles between daughter cells. These SLCs express genes associated with stemness, form mammospheres, and, after transformation, can initiate tumors in vivo (*10*, *11*). Moreover, because of their round morphology, the SLCs can be distinguished by visual inspection from the flat, tightly adherent, nonstemlike mammary epithelial cells with which they coexist in monolayer cultures (Fig. 1B).

To monitor the fate of aged subcellular components, we expressed photoactivatable green fluorescent protein (paGFP) (*12*) in lysosomes, mitochondria, the Golgi apparatus, ribosomes, and chromatin by fusing the fluorescent protein to the appropriate targeting signals or proteins (table S1). paGFP fluoresces only after exposure to a pulse of ultraviolet (UV) light (*12*), allowing us to label each component in a temporally controlled manner (Fig. 1A). Because synthesis of paGFP continues after the light pulse, cells subsequently accumulate unlabeled "young" components in addition to the labeled "old" components; these can be either segregated in distinct subcellular compartments or commingled within individual cells.

We followed the behavior of labeled components in single round SLCs or flat epithelial cells and focused on cell divisions that occurred 10 to 20 hours after paGFP photoactivation (Fig. 1B). The epithelial cells symmetrically apportioned all cellular components analyzed (Fig. 1B). In contrast, the round SLCs apportioned ~5.6 times as much ($P < 0.001$, t test) of ≥10-hour-old mitochondrial outer membrane protein 25 (paGFP-Omp25) to one daughter cell as to the other (Fig. 1B). Similarly, labeled markers for all other organelles examined were apportioned symmetrically. We designated the daughter cell that inherited more aged Omp25 from the mother cell as Progeny1 (P1) and the other as Progeny2 (P2).

To determine whether the same cells that asymmetrically apportion the mitochondrial membrane protein also allocate other membrane compartments asymmetrically, we labeled SLCs with the lipophilic dye PKH26 before photoactivation of paGFP-Omp25. PKH26 initially labels the plasma membrane and is gradually endocytosed to form distinct cytoplasmic puncta, and it is relatively symmetrically apportioned during division of hematopoietic cells (*13*). SLCs apportioned old mitochondria asymmetrically, but the same cells apportioned PKH26 symmetrically (Fig. 1C and movie S1). In contrast, the epithelial cells apportioned both paGFP-Omp25 and PKH26 symmetrically (Fig. 1C and movie S2), similarly to mouse embryonic fibroblasts (not shown).

To verify that SLCs indeed apportion mitochondria according to the age of the organelle, we analyzed the apportioning of paGFP-Omp25 in cell divisions that occurred at random times after the initial photoactivation. We assumed that the age of Omp25 molecules reflected the age of the mitochondria with which they were associated. Cells that divided 0 to 10 hours after photoactivation showed symmetric apportioning of paGFP-Omp25 (Fig. 1D). However, cells that divided more than 10 hours after photoactivation, and thus carried fluorescent marks only on organelles that were at least 10 hours old, apportioned their labeled mitochondria asymmetrically (Fig. 1D).

To follow the apportioning of two different age classes of mitochondria, we tagged mitochondria with mitochondrial proteins fused to a Snap-tag (*14*). Snap-tag is a derivatized DNA repair enzyme, O^6-alkylguanine-DNA alkyltransferase, which can covalently link various fluorophores to the tagged fusion protein in live cells. We used two Snap-tag substrates with two different fluorophores (red

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