4. Z. Guo *et al.*, *Cell Stem Cell* **14**, 188–202 (2013).
5. O. Torper *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 7038–7043 (2013).
6. W. Niu *et al.*, *Nat. Cell Biol.* **15**, 1164–1175 (2013).
7. M. Slezak *et al.*, *Glia* **55**, 1565–1576 (2007).
8. S. Srinivas *et al.*, *BMC Dev. Biol.* **1**, 4 (2001).
9. T. Nomura, C. Göritz, T. Catchpole, M. Henkemeyer, J. Frisén, *Cell Stem Cell* **7**, 730–743 (2010).
10. A. Arvidsson, T. Collin, D. Kirik, Z. Kokaia, O. Lindvall, *Nat. Med.* **8**, 963–970 (2002).
11. J. M. Parent, Z. S. Vexler, C. Gong, N. Derugin, D. M. Ferriero, *Ann. Neurol.* **52**, 802–813 (2002).
12. I. Imayoshi, R. Kageyama, *Neuron* **82**, 9–23 (2014).
13. L. Li *et al.*, *Glia* **58**, 1610–1619 (2010).
14. E. Smith *et al.*, *Genesis* **50**, 700–710 (2012).
15. I. Imayoshi, M. Sakamoto, M. Yamaguchi, K. Mori, R. Kageyama, *J. Neurosci.* **30**, 3489–3498 (2010).
16. A. Varshavsky, *Genes Cells* **2**, 13–28 (1997).
17. L. C. Murtaugh, B. Z. Stanger, K. M. Kwan, D. A. Melton, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14920–14925 (2003).
18. K. Tanigaki *et al.*, *Nat. Immunol.* **3**, 443–450 (2002).
19. A. Ernst *et al.*, *Cell* **156**, 1072–1083 (2014).
20. V. Martínez-Cerdeño *et al.*, *Cell Stem Cell* **6**, 238–250 (2010).

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SUPPLEMENTARY MATERIALS

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CONSERVATION TARGETS

A mid-term analysis of progress toward international biodiversity targets

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In 2010, the international community, under the auspices of the Convention on Biological Diversity, agreed on 20 biodiversity-related "Aichi Targets" to be achieved within a decade. We provide a comprehensive mid-term assessment of progress toward these global targets using 55 indicator data sets. We projected indicator trends to 2020 using an adaptive statistical framework that incorporated the specific properties of individual time series. On current trajectories, results suggest that despite accelerating policy and management responses to the biodiversity crisis, the impacts of these efforts are unlikely to be reflected in improved trends in the state of biodiversity by 2020. We highlight areas of societal endeavor requiring additional efforts to achieve the Aichi Targets, and provide a baseline against which to assess future progress.

Continued degradation of the natural world and the goods and services it provides to humankind has led to the adoption of numerous international agreements aimed at halting the decline of biodiversity and ecosystem services [e.g., (*1*)]. The Parties to the Convention on Biological Diversity (CBD) in 2002 committed to a significant reduction in the rate of biodiversity loss by 2010 (*2*), which, despite some local successes [e.g. (*3*)], did not lead to a reduction in the overall rate of decline (*4*, *5*). Renewed commitments were made in the Strategic Plan for Biodiversity 2011–2020 (*6*), which calls for effective and urgent action this decade. These goals are supported by 20 "Aichi Biodiversity Targets" to be met by 2020 at the latest (table S1), covering "pressures" on, "states" of, and "benefits" from biodiversity and "responses" to the biodiversity crisis [sensu (*4*, *7*); table S2]. Objectively quantifying progress toward these international environmental commitments is critical for assessing their impact and efficacy, yet as the midpoint of this 10-year period approaches, progress toward the Aichi Targets has not been quantitatively evaluated.

To address this gap, we assembled a broad suite of indicator variables to estimate historical trends and project to 2020 (*8*). Building on the CBD's indicative list (*9*), we performed a data scoping of more than 160 potential indicators and reviewed them against five criteria for inclusion, namely: (i) high relevance to a particular Aichi Target and a clear link to the status of biodiversity; (ii) scientific or institutional credibility; (iii) a time series ending after 2010; where unavailable but indicator fills a sizable gap, data ending as near to 2010 as possible; (iv) at least five annual data points in the time series; and (v) broad geographic (preferably global) coverage. Of the 163 potential indicators, 55 met these criteria (table S1), almost double the number used to test whether the 2010 target had been met (*4*). In total, we assembled indicators for 16 of the 20 targets (table S1), and progress to two more was measurable.

We fitted models to estimate underlying trends using an analysis framework adaptive to the highly variable statistical properties of the indicators. Dynamic linear models (*10*) were fitted to high-noise time series, while parametric multimodel averaging (*11*) was used for those with low noise. We projected model estimates and confidence intervals to 2020 to estimate trajectories and rates of change for each indicator (Fig. 1).

As most targets lack explicitly quantifiable definitions of "success" for 2020 (and those that have definitions for some components lack them for others), it was not generally possible to measure progress in terms of distance to a defined end point. Therefore, we assigned indicators as states, pressures, benefits, or responses and compared projected values in 2020 against modeled 2010 values (underlying trend estimates) for all indicators, while additionally measuring absolute progress where possible.

Societal responses to the biodiversity crisis generally showed improvements, with 21 of 33 response indicators (64%) projected to increase significantly by 2020, and most of the remainder having an increasing mean trend. Those increasing significantly included eight of nine indicators of protected area coverage, representativeness, and management (target 11) and all four indicators of sustainable management (fisheries and forest certification, organic farming, and conservation agriculture; targets 6 and 7), along with two of three indicators for research and data provision (Global Biodiversity Information Facility records, research into economic valuation of biodiversity; targets 2 and 19) and two of three indicators of biodiversity awareness (percentage of people who

have heard of biodiversity, percentage correctly defining biodiversity; target 1). However, none of the nine indicators of financial resources showed a significant increase by 2020 (though seven did show positive mean trends), nor did national legislation to prevent or control invasive species.

In contrast, for the underlying state of biodiversity and the pressures upon it, our projections indicate no significant improvement or a worsening situation by 2020, relative to 2010. Five of seven pressure indicators (71%) showed significant increases (a worsening situation), including those measuring consumption (ecological and water footprints, global fishing trawl effort), pollution (nitrogen surplus), and invasive species introductions. Recently emerging pressures (table S5) may also affect outcomes of targets. Among state and benefit indicators, 11 of 17 (65%) showed significant worsening trends, including two indicators of habitat loss (wetland extent and sea ice extent), two of three indicators of population abundance (Farmland Bird Index, Living Planet Index), all six indicators of species extinction risk [an aggregate IUCN Red List Index (RLI) along with disaggregated indices relevant to particular targets], and an indicator of domesticated breeds at risk. We caution, however, against overinterpreting the broader picture for benefits from only three indicators (Fig. 2).

Although some progress is evident across components of individual targets, including targets 1 (awareness), 11 (protected areas), and 19 (knowledge), if biodiversity and ecosystem services are to be maintained and extinction risk averted (targets 12, 13, and 14), additional effort is required to reduce pressures, particularly in relation to targets 4 (sustainable production and consumption), 5 (habitat loss), 8 (pollution), 9 (invasive species), and 10 (climate change impacts) (see fig. S54). For target components with specific numeric goals, we found a mixed picture, where measurable: On current trajectories, the rate of loss of natural habitats (target 5) will not be halved by 2020, all fish stocks will not be sustainably harvested (target 6), and the 10% marine area protection (target 11) will not be met, though taking into account targets set by the parties, actual progress on the latter could exceed extrapolated values (*12*). However, the 17% terrestrial protection component of target 11 is projected to be achieved; target 16 (Nagoya Protocol is in force and operational) and at least part of target 17 (development and adoption of national biodiversity strategy and action plans) are also likely to be met by 2015 (*8*). Although mobilization of financial resources appears to be generally accelerating, our analyses did not detect significant increases by 2020 (target 20); such increases will be needed to support progress toward other targets (*13*).

Comparing the aggregated differences between results for pressure, state, and benefit indicators with those for responses suggests a world in which increasing recognition of the biodiversity crisis is evident, and growing efforts are being made to address it, but one in which the effect of these efforts appears unlikely to be reflected in an improvement in the base state of biodiversity by 2020 (Fig. 2). However, when comparing estimated annual rates of change for each indicator between 2001 to 2010 and 2011 to 2020, our analyses suggest that whereas those for pressure, state, and benefit indicators remain largely unchanged during this period, many response indicators show a positively accelerating rate of change; i.e., a rapid or exponential growth rate (Fig. 3). Although the short post-2010 time span makes it difficult to resolve significant changes in velocity, particularly for financial indicators where there remains large uncertainty, this projected acceleration of response indicators without a comparable signal of their beneficial impacts on biodiversity states, benefits, and pressures by 2020 could be due to several factors. One

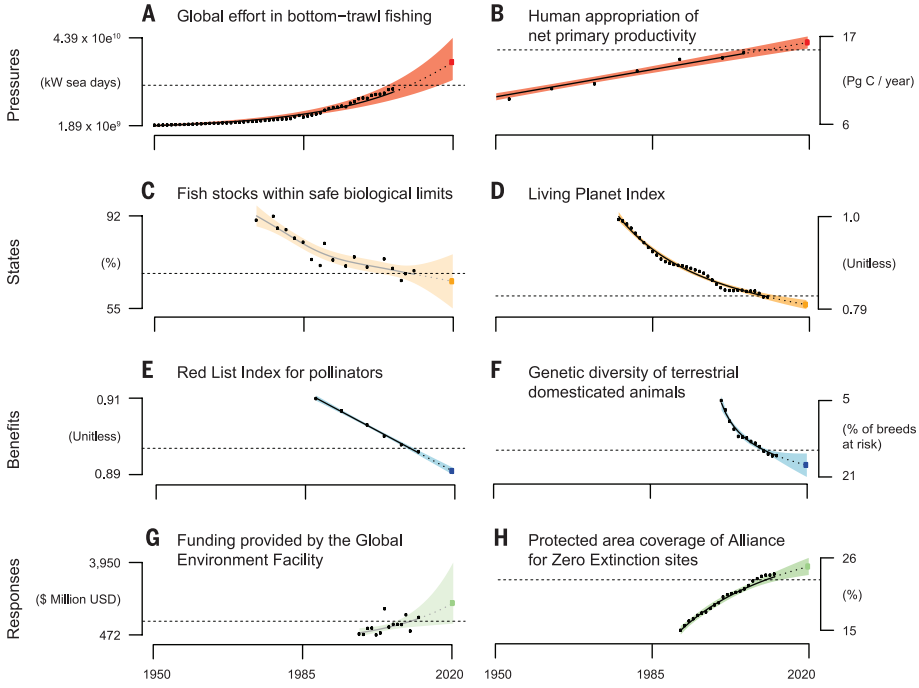

Fig. 1. Examples of model fits and projections for indicator data. Panels show selected pressure (**A** and **B**), state (**C** and **D**), benefit (**E** and **F**), and response (**G** and **H**) indicator data (black dots). Model fits (black and gray lines) and 95% confidence intervals (dark and light shading) indicate, respectively, significant and non-significant differences between 2010 (horizontal dashed line) and 2020 (colored square) estimates. (A) and (B) have been truncated at 1950 for visualization purposes. For fits to all 55 indicator time series, see fig. S54.

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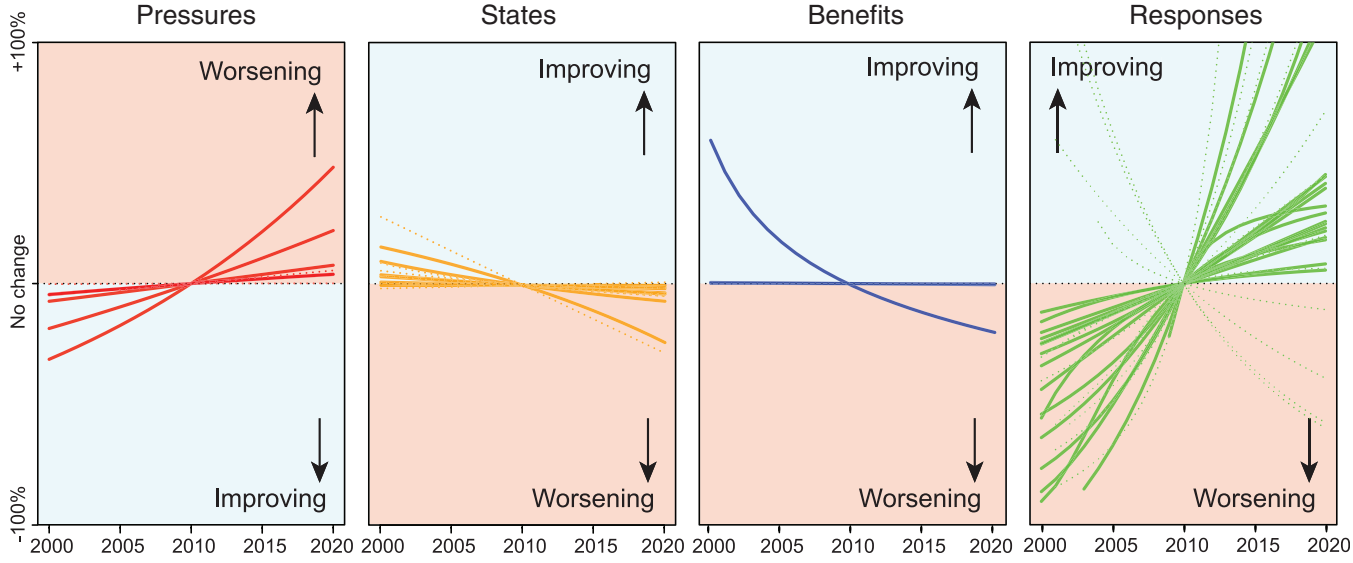

Fig. 2. Aggregated trends in pressures, states, benefits, and responses across all indicators and Aichi Targets. Lines represent significant (continuous) or nonsignificant (dotted) trends relative to 2010 modeled value (horizontal dotted black line). Indicators with very flat linear trends may be superimposed (e.g., two benefit indicators). An increase in states, benefits, and responses, or a decrease in pressures represents progress toward the targets. Some indicator trends (e.g., extinction rates) have been inverted to conform to this paradigm. Trends have been truncated before 2000 for visualization purposes.

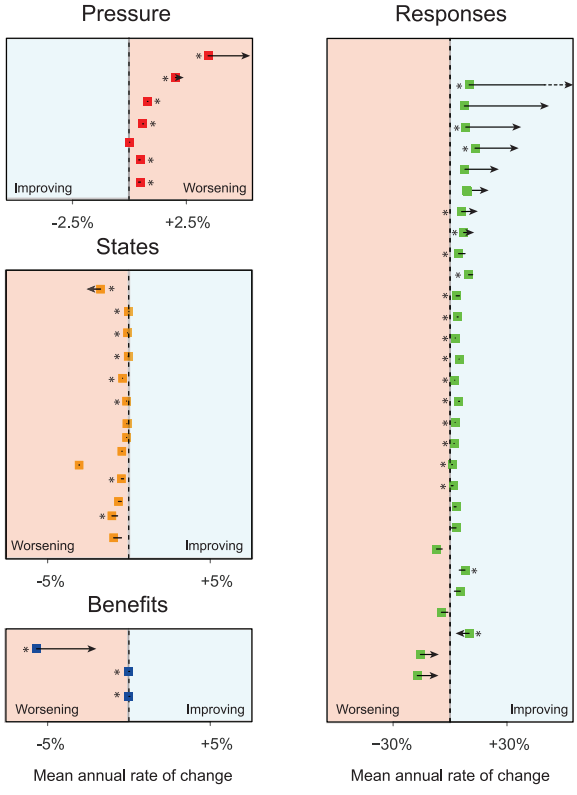

Fig. 3. Comparison between mean annual rates of change in indicators pre- and post-2010. Filled squares indicate estimated mean annual rate of change of indicator between 2001 (or earliest year if subsequent) and 2010. End points of arrows indicate estimated mean annual rate of change between 2011 and 2020. Indicators to the right of the vertical dashed line are increasing annually, whereas those to the left are decreasing. If arrows point toward the dashed line, then rate of change is slowing; conversely, if they point away, it is accelerating. Black asterisks indicate significant slopes for post-2010 mean rates of change, based on bootstrapped linear model fits. Dashed arrow indicates value beyond *x*-axis limit. Two state indicators for target 1 have been excluded because they only have a single year before 2010. For identification of each indicator, see table S8.

possibility is that there are substantial time lags before outcomes are detectable. That is, it may take years or decades before these increased responses translate to positive changes in the state of biodiversity or reduced pressures (*14*). Ecological theory and restoration ecology provide tangible evidence that supports this assertion (*15*–*17*), and a notable escalation of responses as implied here may signal improved progress toward targets over longer time scales; indeed, state, benefit, and pressure indicators already implicitly reflect prior conservation action. Alternatively, responses may be insufficient or inappropriate relative to pressures and fail to overcome the growing impacts of drivers that lead to biodiversity loss.

It is important to recognize that statistical extrapolations make the assumption of underlying processes remaining constant into the future, which may or may not hold, and should be viewed with this assumption clearly in mind. Our analyses are also inevitably incomplete. A global analysis will not reflect finer-scale spatial variation and local to regional improvements [e.g., (*3*, *18*)], and the taxonomic coverage is limited. Locating data that enable quantification of progress toward targets at a global scale is challenging (*19*, *20*), and some indicators are less well aligned with targets, leading to variable levels of coverage (fig. S53 and tables S3 and S4) (*21*). Indicators also have differing spatial, temporal, and/or taxonomic coverage (table S1), and for some individual target components (e.g., harmful subsidies for target 3, plant genetic resources for target 13), we were unable to locate indicators satisfying our criteria (table S3). Moreover, we could not locate any indicators meeting the criteria above to measure progress toward targets 15 (ecosystem resilience and contribution of biodiversity to carbon stocks) and 18 (integration of traditional knowledge and effective participation of indigenous and local communities). Investment in the development of novel indicators for unassessed targets or components remains an urgent priority, as does the development of indicators for "benefits" from ecosystems (*7*), of which we could only locate three. Novel data collection, data-sharing platforms, and support to developing nations in analytical capacities and training may help contribute to these goals, as may contemporary approaches to assessing the impact of interventions (*22*).

Despite these limitations, the rapid development of online databases, indicators, and indicator partnerships continues to improve our

ability to quantify progress toward targets (*23*). The benefits of maintaining biodiversity are well known (*24*). Our results provide a baseline against which to measure progress toward this objective in 2020 and suggest that efforts need to be redoubled to positively affect trajectories of change and enable global biodiversity goals to be met by the end of the current decade.

REFERENCES AND NOTES

1. United Nations, *Convention on Biological Diversity* (Rio de Janeiro, Brazil, 1992).
2. Secretariat of the Convention on Biological Diversity (SCBD), "Handbook of the Convention on Biological Diversity" (Earthscan, London, 2003).
3. B. Worm *et al*., *Science* **325**, 578–585 (2009).
4. S. H. M. Butchart *et al*., *Science* **328**, 1164–1168 (2010).
5. SCBD, "Global Biodiversity Outlook 3" (Montreal, 2010).
6. UNEP, CBD, "UNEP/CBD/COP/DEC/X/2 2010" (2010).
7. T. H. Sparks *et al*., *Oryx* **45**, 411–419 (2011).
8. See supplementary materials on *Science* Online.
9. UNEP, CBD, UNEP/CBD/COP/DEC/XI/3 2012 (2012).
10. J. Durbin, S. J. Koopman, *Time Series Analysis by State Space Methods* (Oxford Univ. Press, Oxford, 2001).
11. K. P. Burnham, D. R. Anderson, *Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach* (Springer, New York, ed. 2, 2002).
12. CBD, Programme of Work on Protected Areas (PoWPA), https://www.cbd.int/protected/implementation/actionplans/ (2013).
13. D. P. McCarthy *et al*., *Science* **338**, 946–949 (2012).
14. Furthermore, the ability of statistical models to react to recent changes in indicator trends will vary depending on, among other things, the length of the time series, the noise in the data, and the magnitude of departure from the previous trend.
15. J. M. Bullock, J. Aronson, A. C. Newton, R. F. Pywell, J. M. Rey-Benayas, *Trends Ecol. Evol.* **26**, 541–549 (2011).
16. J. P. Metzger *et al*., *Biol. Conserv.* **142**, 1166–1177 (2009).
17. M. Di Marco *et al*., *Conserv. Biol.* **28**, 1109–1118 (2014).
18. D. Nepstad *et al*., *Science* **344**, 1118–1123 (2014).
19. M. Walpole *et al*., *Science* **325**, 1503–1504 (2009).
20. H. M. Pereira, L. M. Navarro, I. S. Martins, *Annu. Rev. Environ. Resour.* **37**, 25–50 (2012).
21. It is also possible that the response, pressure, and state indicator framework is not tracking factors that are causally linked (*7*); use of this framework does not imply joined-up indicators.
22. M. Hoffmann *et al*., *Science* **330**, 1503–1509 (2010).
23. S. L. Pimm *et al*., *Science* **344**, 1246752 (2014).
24. B. J. Cardinale *et al*., *Nature* **486**, 59–67 (2012).

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SUPPLEMENTARY MATERIALS

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CELL-FREE ASSAYS

Spatial organization of cytokinesis signaling reconstituted in a cell-free system

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During animal cell division, the cleavage furrow is positioned by microtubules that signal to the actin cortex at the cell midplane. We developed a cell-free system to recapitulate cytokinesis signaling using cytoplasmic extract from *Xenopus* eggs. Microtubules grew out as asters from artificial centrosomes and met to organize antiparallel overlap zones. These zones blocked the interpenetration of neighboring asters and recruited cytokinesis midzone proteins, including the chromosomal passenger complex (CPC) and centralspindlin. The CPC was transported to overlap zones, which required two motor proteins, Kif4A and a Kif20A paralog. Using supported lipid bilayers to mimic the plasma membrane, we observed the recruitment of cleavage furrow markers, including an active RhoA reporter, at microtubule overlaps. This system opens further approaches to understanding the biophysics of cytokinesis signaling.

Actomyosin-based cleavage furrows in animal cells are positioned by signals emanating from microtubule assemblies formed shortly after anaphase onset (*1*). In typical somatic cells, the signaling complexes centralspindlin and the chromosomal passenger complex (CPC) accumulate at the center of the midzone (or central spindle), which forms in the space previously occupied by the mitotic spindle (*2*). It is unclear how the microtubules that position furrows are organized in much larger egg cells and how they signal to the cortex. We addressed these questions by developing a cell-free system to reconstitute the spatial signaling that is characteristic of cytokinesis in a large egg cell.

To reconstitute cytokinesis events, undiluted egg cytoplasm with intact actin (*3*), containing fluorescent probes and Aurora kinase A (AurkA)–based artificial centrosome beads (*4*), was treated with Ca^{2+} to mimic fertilization and immediately spread between two coverslips for imaging (fig. S1A). As the cell cycle progressed from metaphase to interphase (*5*), large microtubule asters grew out rapidly from each AurkA bead. Where the expanding edges of two neighboring asters met, antiparallel microtubule bundles formed in a boundary zone that we term the aster-aster interaction zone (AAIZ) (Fig. 1, A to C, fig. S1, and movie S1). In somatic cells, the CPC and centralspindlin complexes are recruited to the midplane in anaphase, where they specify the division plane by activating the small GTPase RhoA (*2*). We imaged endogenous complexes by adding labeled antibodies, and for the CPC we confirmed localization with a green fluorescent protein (GFP)–tagged DasraA subunit (*5*). CPC and the Kif23 subunit of centralspindlin were recruited to the AAIZ in a 5- to 15-μm-wide line bisecting the line between two AurkA beads (Fig. 1, B and C, and fig. S1). The AAIZ was wider than a somatic cell midzone and was hundreds of microns long. To evaluate its physiological relevance, we imaged the same proteins in *Xenopus* zygotes fixed between mitosis and cytokinesis, which takes place at interphase in early embryonic cells (Fig. 1D) (*6*). The morphology of the midplane in zygotes, as defined by microtubule morphology and CPC/centralspindlin localization, was strikingly similar to that of the AAIZ in extracts (Fig. 1, A to C).

To measure microtubule orientation at the AAIZ we tracked GFP-tagged end-binding protein 1 (EB1), which binds to growing microtubule plus ends (Fig. 1E, fig. S2, and movie S2) (*7*). Microtubules grew outward radially within each aster. At the AAIZ, EB1 comets from both directions entered antiparallel bundles, where they usually disappeared (Fig. 1E). We quantified the degree of interpenetration by categorizing EB1 comets based on their direction (fig. S3) (*5*). The AAIZ was characterized by a sharp change in directionality over ~20 μm, indicating a localized block to interpenetration between the asters (Fig. 1F).

Kinase activity of the Aurora kinase B (AurkB) subunit of the CPC is required to establish midzone morphology and for furrow ingression (*8*). We confirmed this in *Xenopus* eggs (fig. S4) (*5*). AurkB inhibition blocked recruitment of the CPC in our cell-free system (Fig. 1E) and caused much deeper interpenetration of microtubules (Fig. 1, E and F, and movie S3). Thus, AurkB activity was required to create a sharp boundary between asters.

CPC is proposed to be transported to the center of midzones along microtubules by a kinesin molecular motor (*9*), but transport has not been observed directly. Five plus-end–directed kinesins involved in cell division are candidates for CPC transport (*10*): Kif4A, Kif10 (also called CenpE),

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