

REPORTS

in antibody levels [exceeding the ~20-day half-life of human IgG (19)] are consistent with the generation of long-lived plasma cells that turn over with a half-life of ~40 days.

Taken together, the above results indicate that memory B cells have two response modes. In the antigen-dependent mode, they undergo a massive expansion and differentiation toward short-lived plasma cells. This response is transient, because of the negative feedback exerted by the high level of antibody present (20). However, some plasma cells generated in this way become long-lived if rescued in available niches such as bone marrow (21). These cells sustain serum antibody levels, but can do so only for a few months, because of their limited life-span. In contrast, in the polyclonal mode, all memory B cells respond to environmental stimuli by undergoing continuous proliferation and differentiation. In this way, a constant level of plasma cells and serum antibodies could theoretically be maintained throughout a human life-span. Because this mechanism is non-specific, it would act indiscriminately to maintain the broad spectrum of antibody specificities generated during the antigen-driven immune response.

The sensitivity to polyclonal stimuli represents a key feature of human memory B cells and adds a novel property to the concept of "memory stem cells" (22, 23). The differential response to polyclonal stimuli may reflect distinct roles of switch and IgM memory B cell subsets. Thus, although the exquisite sensitivity of switch memory B cells to bystander help may be instrumental in maintaining systemic IgG antibody levels, the capacity of IgM memory B cells to respond to CpG in the absence of cytokines could be instrumental in maintaining levels of natural antibodies to bacterial antigens (24–26). This may also underline the propensity to develop mucosa-associated lymphatic tissue (MALT) lymphomas under chronic infection by *Helicobacter pylori* (27). It is possible that polyclonal stimuli in addition to CpG or T cell help activate memory B cells in vivo within the microenvironment of secondary lymphoid organs or of the bone marrow, where memory B cells and proliferating plasma cell precursors are present (28, 29).

Our results discriminate between a "short-term serological memory," which is antigen-dependent and lasts for a few months, and a "long-term serological memory" that results from an antigen-independent polyclonal activation and differentiation of memory B cells. The possibility of selectively targeting one component or the other may open new ways for effective vaccination.

References and Notes

- K. Rajewsky, *Nature* **381**, 751 (1996).
- J. Banchereau et al., *Annu. Rev. Immunol.* **12**, 881 (1994).
- M. Maruyama, K. P. Lam, K. Rajewsky, *Nature* **407**, 636 (2000).
- R. A. Manz, A. Thiel, A. Radbruch, *Nature* **388**, 133 (1997).
- M. K. Slifka, R. Antia, J. K. Whitmire, R. Ahmed, *Immunity* **8**, 363 (1998).
- R. Ahmed, D. Gray, *Science* **272**, 54 (1996).
- R. A. Manz et al., *Curr. Opin. Immunol.* **14**, 517 (2002).
- R. M. Zinkernagel, *Curr. Opin. Immunol.* **14**, 523 (2002).
- A. F. Ochsenbein et al., *Proc. Natl. Acad. Sci. U.S.A.* **97**, 13263 (2000).
- A. Poltorak et al., *Science* **282**, 20 (1998).
- A. M. Krieg, *Annu. Rev. Immunol.* **20**, 709 (2002).
- A. Lanzavecchia, *Eur. J. Immunol.* **13**, 820 (1983).
- U. Klein, K. Rajewsky, R. Kuppers, *J. Exp. Med.* **188**, 1679 (1998).
- See supporting data on Science Online.
- N. L. Bernasconi, E. Traggiai, unpublished data.
- K. M. Ansel et al., *J. Exp. Med.* **190**, 1123 (1999).
- M. Odendahl et al., *J. Immunol.* **165**, 5970 (2000).
- These observations may explain why a correlation between serum antibodies and antigen-binding memory B cells was not detected in a previous study where recently boosted individuals were analyzed (30).
- A. Morell, W. D. Terry, T. A. Waldmann, *J. Clin. Invest.* **49**, 673 (1970).
- J. W. Uhr, G. Moller, *Adv. Immunol.* **8**, 81 (1968).
- R. A. Manz, A. Radbruch, *Eur. J. Immunol.* **32**, 923 (2002).
- F. Celada, *J. Exp. Med.* **125**, 199 (1967).
- D. T. Fearon, P. Manders, S. D. Wagner, *Science* **293**, 248 (2001).
- S. Fagarasan, T. Honjo, *Science* **290**, 89 (2000).
- A. J. Macpherson et al., *Science* **288**, 2222 (2000).
- F. Martin, J. F. Kearney, *Nature Rev. Immunol.* **2**, 323 (2002).
- R. Kuppers, U. Klein, M. L. Hansmann, K. Rajewsky, *N. Engl. J. Med.* **341**, 1520 (1999).
- E. Paramithiotis, M. D. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 208 (1997).
- B. P. O'Connor, M. Cascalho, R. J. Noelle, *J. Exp. Med.* **195**, 737 (2002).
- H. Leyendeckers et al., *Eur. J. Immunol.* **29**, 1406 (1999).
- We thank J. C. Howard, K. Karjalainen, G. Natoli, and F. Sallusto for critical reading of the manuscript. A.L. is supported by the Helmut Horten Foundation and by the Swiss National Science Foundation (grant 31-63885).

Supporting Online Material

www.sciencemag.org/cgi/content/full/298/5601/2199/DC1

Materials and Methods

Table S1

References and Notes

12 July 2002; accepted 7 October 2002

Rainfall Variability, Carbon Cycling, and Plant Species Diversity in a Mesic Grassland

Alan K. Knapp,^{1*} Philip A. Fay,¹ John M. Blair,¹ Scott L. Collins,^{1,2} Melinda D. Smith,³ Jonathan D. Carlisle,¹ Christopher W. Harper,¹ Brett T. Danner,¹ Michelle S. Lett,¹ James K. McCarron¹

Ecosystem responses to increased variability in rainfall, a prediction of general circulation models, were assessed in native grassland by reducing storm frequency and increasing rainfall quantity per storm during a 4-year experiment. More extreme rainfall patterns, without concurrent changes in total rainfall quantity, increased temporal variability in soil moisture and plant species diversity. However, carbon cycling processes such as soil CO₂ flux, CO₂ uptake by the dominant grasses, and aboveground net primary productivity (ANPP) were reduced, and ANPP was more responsive to soil moisture variability than to mean soil water content. Our results show that projected increases in rainfall variability can rapidly alter key carbon cycling processes and plant community composition, independent of changes in total precipitation.

Anthropogenic climate change is projected to include increasingly variable precipitation regimes, as well as atmospheric warming (1). General circulation models forecast a higher frequency of extreme rainfall events from intense convective storms, a lower frequency of rainfall days, and longer intervening dry

periods (1–5). Evidence is mounting that an increase in precipitation extremes has begun to occur worldwide (5–8). Most aspects of terrestrial ecosystem structure and function are vulnerable to these hydrologic changes, perhaps independent of changes in annual precipitation quantity (1, 9, 10), and important interactions with elevated temperatures and atmospheric carbon dioxide can be expected (11). Thus, models and policy analyses of the consequences of climate change should not rely on scenarios that focus primarily on climatic means (12, 13). Yet, our ability to forecast ecosystem responses to climate change is constrained by a lack of

¹Division of Biology, Kansas State University, Manhattan, KS 66506, USA. ²Ecological Studies Program, National Science Foundation, Arlington, VA 22230, USA. ³National Center for Ecological Analysis and Synthesis, University of California, Santa Barbara, CA 93101, USA.

*To whom correspondence should be addressed. E-mail: aknapp@ksu.edu

REPORTS

field studies and proxy data sets capable of projecting the long-term consequences of increased climatic variability.

To quantify the impact of increased intra-annual rainfall variability on an intact ecosystem, we altered the temporal distribution and size of rainfall events, without changing total precipitation amounts, for four growing seasons in a native grassland. Here we examine responses in C cycling and plant community composition, which are key ecosystem attributes with the potential to impart feedback on climate change (9). Grasslands are ideal for such experiments because they are extensive biomes with large C storage capacity, are often highly productive and species-rich, and are among the most responsive of terrestrial ecosystems to interannual variability in precipitation (14–16).

In a native grassland ecosystem in northeast Kansas, United States, where C_4 perennial grasses dominate in biomass and cover (14), we manipulated rainfall variability and reduced rainfall quantity in 12 rainfall manipulation plots (RaMPs) from 1998 to 2001 (17, 18). For this analysis, we focus only on responses to the increased rainfall variability treatment. Rainfall patterns were altered relative to control (ambient) treatments by extending the intervals between natural rain events by 50%, collecting and storing all rain falling during these intervals, and then applying the accumulated quantities as single large events. As a result, control plots received 25 to 30 rain events based on actual rainfall patterns during each growing season (mean per event = 14 mm), whereas plots exposed to altered rainfall patterns received fewer but larger events (6 to 8 per growing season; mean per event = 42 mm), with longer dry periods between storms and more total dry days (Fig. 1). The large size and low frequency of rainfall events in the altered precipitation treatment are well within the range of documented rainfall regimes of the past 100 years in this region (18). Thus, rainfall variability was increased without imposing unrealistic drought periods or extraordinary storm intensities. These manipulations allowed us to assess responses of this grassland to the predicted rainfall regime of more intense storms and an increased number of dry days during the growing season (3, 4), without the confounding effects of altered total precipitation inputs (Fig. 1).

Temporal patterns of soil water content were strongly influenced by increased rainfall variability (Fig. 1). Over the 4-year study, average soil water content in the upper 30 cm, where >70% of root biomass occurs in this and in most grasslands (19, 20), was reduced by 11.6% [$F(1,8) = 31.00, P = 0.0005$] in altered versus ambient rainfall plots (Fig. 1). Reduced mean soil water content was caused

by more frequent and prolonged periods of low soil moisture due to extended intervals between rainfall inputs. We used an unbiased distance function, the mean absolute difference in soil water content between consecutive measurements (21), to quantify temporal dynamics in soil moisture. This estimate of soil moisture variability increased by 27% [$F(1,8) = 21.4, P = 0.002$] across all years in plots exposed to altered rainfall patterns, with increases as much as twofold in some years (Fig. 1B, inset). Thus, stability in soil moisture supply decreased as precipitation variability increased.

Increased variability in rainfall and soil water content significantly affected three key C cycling processes in this grassland (17). Midsummer net photosynthesis (leaf-level CO_2 uptake) by the dominant grass, *Andropogon gerardii*, was reduced ~20% in plants

subjected to increased rainfall variability, consistent with increased leaf-level water stress at midseason (14% lower midday leaf water potential) (Fig. 2A). This is a critical period in the growing season when this dominant species ($79.4 \pm 3.2\%$ canopy cover) achieves maximum vegetative growth, initiates flowering, and produces seed (14). Over the 4-year experiment, exposure to fewer but larger rainfall events reduced aboveground net primary production (ANPP) by ~10% as compared to ambient rainfall patterns (Fig. 2A), with the greatest reduction in ANPP (107.9 g/m^2) occurring during the driest year of the study, when annual precipitation was 22% below the long-term average (835 mm). The majority (86%) of this decline in ANPP was due to reduced productivity of the C_4 grasses. Thus, reduced CO_2 uptake by the dominant grass, *A. gerardii*, provides a

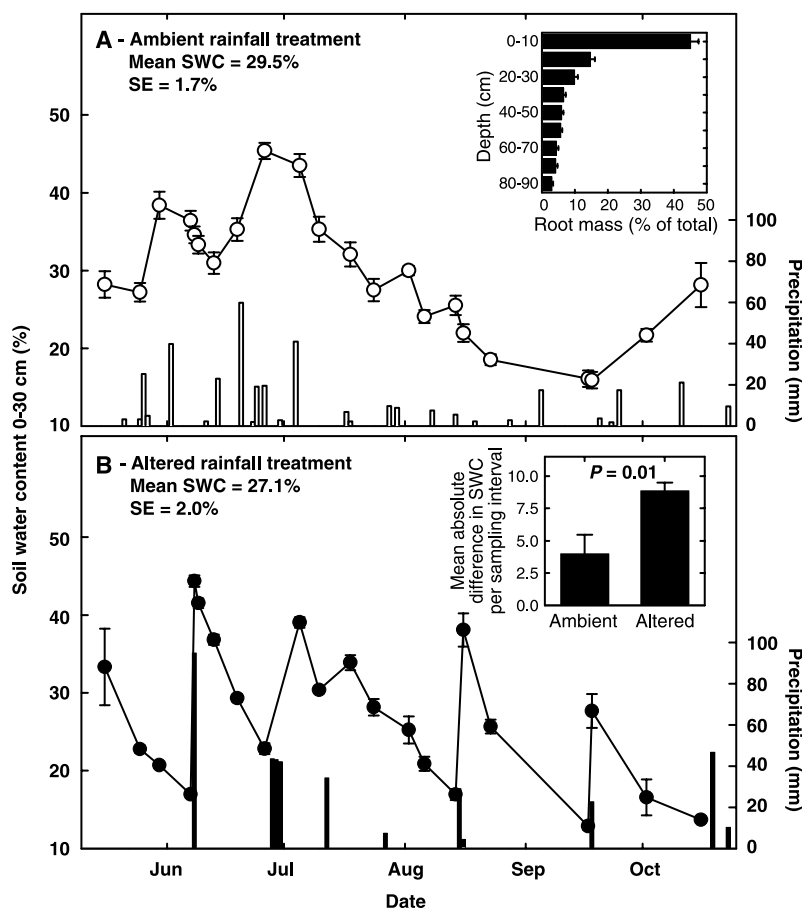


Fig. 1. Seasonal dynamics of volumetric soil water content (SWC) integrated over the upper 30 cm of the soil during the 2000 growing season in a C_4 -dominated grassland in NE Kansas, United States. SWC was measured in plots in the RaMPs facility (17, 18) that (A) received precipitation inputs identical to ambient patterns or (B) were exposed to an altered rainfall regime in which dry intervals between rain events were extended and individual storms were larger (compare precipitation patterns at the bottom of each panel). Although total precipitation inputs were the same for each treatment [growing season (April to September) rainfall averaged 615 mm over the 4-year study], mean SWC was lower [$F(1,8) = 42.34, P = 0.0002$] in the altered treatment than in the ambient treatment in the year shown. SE, standard error of the mean. In (A), the inset shows mean root biomass distribution by depth measured in 2001 for all experimental plots combined. There was no significant treatment effect on root depth distribution or biomass. In (B), the inset shows that the mean absolute difference in SWC between consecutive measurements, an estimate of the temporal dynamics of SWC (21), was significantly higher in altered than in ambient plots.

REPORTS

mechanistic link among alterations in resource availability, increased plant stress, and subsequent ecosystem-level responses.

The strong and direct effect of increased rainfall variability on C inputs to this ecosys-

tem was further illustrated by comparing the relationships between ANPP and mean soil water content, and ANPP and variability in soil moisture (Fig. 2). Despite growing-season precipitation amounts that varied twofold

over the 4-year study, ANPP was not related to mean soil water content (Fig. 2B). However, ANPP was strongly and negatively related to the temporal variability in soil water content (Fig. 2C), which is evidence that ecosystem C inputs from ANPP can be directly affected by altered rainfall variability, independent of precipitation quantity.

Grassland soils are among the most important globally for long-term C storage (16). Soil CO₂ flux, an index of belowground plant and microbial heterotrophic activity, was reduced significantly (16%) when rainfall variability was increased (Fig. 2A). The magnitude of this effect was two times as great as the responses to increased air and soil temperatures (2.0° to 2.6°C) measured previously in a similar grassland (22). Although reduced soil CO₂ flux may decrease overall ecosystem C loss in the short term, it is also indicative of lower belowground productivity (23, 24). When coupled with reduced productivity aboveground, the results suggest that increased rainfall variability will decrease C inputs overall and potentially reduce long-term soil C sequestration.

Increased variability in precipitation enhanced plant community diversity (Shannon's index, *H'*) (Fig. 3, inset) and was accompanied by increased turnover of rare and uncommon species (33 compared to 24 cumulative colonization and extinction events in altered and ambient plots, respectively). Increased diversity could be a direct response of the plant community to increased soil moisture dynamics (Fig. 3A) (25), but *H'* was also negatively related to ANPP in this experiment (Fig. 3B). Thus, the plant community could be responding indirectly to reduced ANPP, a commonly observed pattern in natural ecosystems (26). The increases in diversity, measured at both broad and fine scales, were due to increased richness and evenness (table S1), suggesting that longer-term shifts in community composition will occur as more extreme precipitation regimes chronically alter both the mean and temporal variability of soil moisture.

Linking short-term responses to ecosystem properties that develop over longer time scales can extend the inference of this experiment. In this mesic grassland, drier and more variable temporal patterns of soil moisture occur in upland relative to lowland sites (27, 28). Increased variability in soil moisture in uplands is due to the combined effects of soil textural differences, higher evaporative demand, and more rapid soil water drainage relative to lowlands (14) rather than to differences in precipitation patterns. Nonetheless, these sites may provide insight for projecting, at least qualitatively, potential long-term effects of increased precipitation variability in this grassland. Results from a variety of Konza Prairie Long-Term Ecolog-

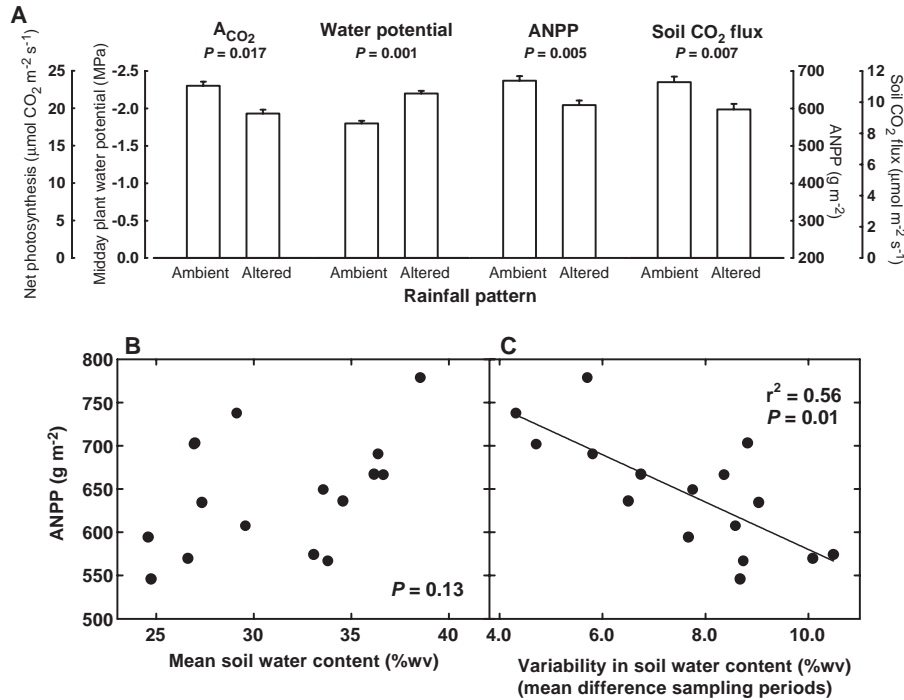


Fig. 2. (A) Responses in three key C cycling processes to increased rainfall variability (altered) versus ambient rainfall patterns (Fig. 1). These processes include (i) midday CO₂ uptake (net photosynthesis, A_{CO_2}) by the dominant grass, *A. gerardii*, shown with an estimate of plant water stress (midday water potential); (ii) ANPP as an integrator of net C uptake by all species; and (iii) seasonal mean soil CO₂ flux, an indicator of belowground plant and microbial respiration. Responses in A_{CO_2} and water potential were measured in August of 1998–1999 only, whereas ANPP and soil CO₂ flux were measured in all 4 years of the experiment. Statistical significance for paired comparisons is indicated above the bars. Complete statistical analyses are also available (table S1). Relationship between ANPP and mean SWC during the growing season (B) and ANPP and variability in SWC (C), calculated as the mean absolute difference in soil water content between measurements (Fig. 1B). These relationships are based on 4 years of rainfall manipulation, and they include all plots in the experimental design (four treatments per year) (17).

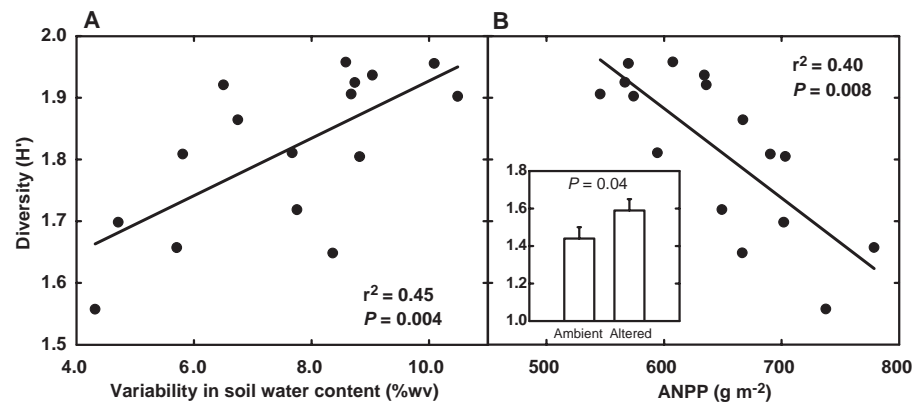


Fig. 3. Relationship between plant species diversity (Shannon's index, *H'*) and variability in soil water content (Fig. 2C) (A) and *H'* and ANPP (B). Data are combined from 4 years of ambient and altered rainfall treatments and include all plots in the experimental design [four treatments per year (17)]. Broad-scale *H'* was calculated by averaging relative abundance (cover; *n* = 3 plots) of each species for each treatment. (Inset) Fine-scale response in *H'* to increased (altered) versus ambient rainfall variability. For this analysis, *H'* was calculated by averaging relative abundance (stem density) of each species from four 0.1-m² subplots per RaMP in 2001. Analyses of diversity components, richness, and evenness are also provided in table S1.

REPORTS

ical Research (LTER) studies (14, 23, 27, 28) comparing C cycling and community attributes in upland and lowland sites (table S2) are consistent with the responses to rainfall variability reported in this 4-year experiment. Thus, reduced soil C storage and trace gas fluxes, reduced dominance by productive C₄ grasses, and a more diverse and xeric flora, all characteristic of upland sites (14), can be forecast as longer-term and more widespread responses to chronic increases in rainfall variability in this mesic grassland.

Our results have several implications for predicting the magnitude and direction of change in grasslands under future climates. First, it is clear that species, community, and ecosystem responses to alterations in storm frequency and intensity can be rapid and independent of changes in annual precipitation amount. Increased temporal variability in precipitation patterns and soil moisture in grasslands will, in the short term, increase plant water stress and alter key C cycling processes such as net photosynthesis, aboveground productivity, and soil CO₂ flux, with long-term consequences for C storage and biotic-atmospheric feedbacks. Shifts in plant species composition toward more diverse communities and toward communities characteristic of more xeric environments are also expected, further lowering productivity without a change in mean rainfall quantity. Second, future climates are predicted to include changes both in precipitation variability and in quantity (1, 5, 7, 10). Thus, projected ecosystem responses to wetter or drier rainfall regimes must account for concomitant changes in storm frequency and size (10). In this study, responses to increased rainfall variability were greatest in the driest years, suggesting that greater impacts will occur in concert with drought or in more arid systems. Finally, 90% of all terrestrial vegetation types worldwide have >50% of their roots in the upper 0.3 m of the soil profile (19, 20). Consequently, most biomes are at risk of being affected by projected increases in rainfall variability and more extreme soil moisture dynamics, independent of changes in precipitation quantity.

References and Notes

- Intergovernmental Panel on Climate Change, *Climate Change 2001: Synthesis Report, Third Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge Univ. Press, New York, 2001).
- D. Rind, R. Goldberg, R. Ruedy, *Clim. Change* **14**, 5 (1989).
- L. O. Mearns, S. H. Schneider, S. L. Thompson, L. R. McDaniel, *J. Geophys. Res.* **95**, 20469 (1990).
- H. B. Gordon, P. H. Whetton, A. B. Pittock, A. M. Fowler, M. R. Haylock, *Clim. Dyn.* **8**, 83 (1992).
- D. R. Easterling et al., *Science* **289**, 2068 (2000).
- T. R. Karl, R. W. Knight, N. Plummer, *Nature* **377**, 217 (1995).
- P. Y. Groisman et al., *Clim. Change* **42**, 243 (1999).
- N. Plummer et al., *Clim. Change* **42**, 183 (1999).
- D. M. Gates, *Climate Change and Its Biological Consequences* (Sinauer, Sunderland, MA, 1993).
- L. O. Mearns, C. Rosenzweig, R. Goldberg, *Clim. Change* **35**, 367 (1997).
- G. W. Koch, H. A. Mooney, *Carbon Dioxide and Terrestrial Ecosystems* (Academic Press, New York, 1996).
- R. W. Katz, B. G. Brown, *Clim. Change* **21**, 289 (1992).
- C. Parmesan, T. L. Root, M. R. Willig, *Bull. Am. Meteorol. Soc.* **81**, 443 (2000).
- A. K. Knapp, J. M. Briggs, D. C. Hartnett, S. L. Collins, *Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie* (Oxford Univ. Press, New York, 1998).
- A. K. Knapp, M. D. Smith, *Science* **291**, 481 (2001).
- W. H. Schlesinger, *Biogeochemistry: An Analysis of Global Change* (Academic Press, New York, 1997).
- Materials and methods are available as supporting material on Science Online.
- P. A. Fay, J. D. Carlisle, A. K. Knapp, J. M. Blair, S. L. Collins, *Ecosystems* **3**, 308 (2000).
- R. B. Jackson et al., *Oecologia* **108**, 389 (1996).
- J. J. Schenk, R. B. Jackson, *Ecol. Monogr.* **72**, 311 (2002).
- J. A. Ludwig, J. F. Reynolds, *Statistical Ecology* (Wiley, New York, 1988).
- Y. S. Luo, S. Wan, D. Hui, L. L. Wallace, *Nature* **413**, 622 (2001).
- A. K. Knapp, S. L. Conard, J. M. Blair, *Ecol. Appl.* **8**, 760 (1998).
- L. C. Johnson, J. R. Matchett, *Ecology* **82**, 3377 (2001).
- R. E. Ricklefs, *Am. Nat.* **111**, 376 (1977).
- R. B. Waide et al., *Annu. Rev. Ecol. Syst.* **30**, 257 (1999).
- D. S. Schimel et al., *Ecology* **72**, 672 (1991).
- A. K. Knapp et al., *Ecology* **74**, 549 (1993).
- We thank P. Reich and R. Jackson for providing valuable comments on an earlier version of this manuscript. Supported by USDA Ecosystems, U.S. Department of Energy National Institute for Global Environmental Change and the NSF LTER programs, and the Nature Conservancy.

Supporting Online Material

www.sciencemag.org/cgi/content/full/298/5601/2202/DC1

Materials and Methods
Tables S1 and S2

19 July 2002; accepted 21 October 2002

Small Nuclear Ribonucleoprotein Remodeling During Catalytic Activation of the Spliceosome

Evgeny M. Makarov,¹ Olga V. Makarova,¹ Henning Urlaub,¹
Marc Gentzel,² Cindy L. Will,¹ Matthias Wilm,²
Reinhard Lührmann^{1*}

Major structural changes occur in the spliceosome during its activation just before catalyzing the splicing of pre-messenger RNAs (pre-mRNAs). Whereas changes in small nuclear RNA (snRNA) conformation are well documented, little is known about remodeling of small nuclear ribonucleoprotein (snRNP) structures during spliceosome activation. Here, human 45S activated spliceosomes and a previously unknown 35S U5 snRNP were isolated by immunoaffinity selection and were characterized by mass spectrometry. Comparison of their protein components with those of other snRNP and spliceosomal complexes revealed a major change in protein composition during spliceosome activation. Our data also suggest that the U5 snRNP is dramatically remodeled at this stage, with the Prp19 complex and other factors tightly associating, possibly in exchange for other U5 proteins, and suggest that after catalysis the remodeled U5 is eventually released from the postslicing complex as a 35S snRNP particle.

Pre-mRNA splicing, the removal of introns from mRNA precursors, is a prerequisite for the expression of most eukaryotic genes. Catalysis of the two transesterification steps of the pre-mRNA splicing reaction takes place in the spliceosome, an elaborate molecular machine formed by the ordered interaction of four small nuclear ribonucleoproteins (snRNPs), U1, U2, U5, and U4/U6, and numerous splicing factors with the pre-mRNA (1). Spliceosome assembly is initiated by the interaction of the U1 and U2 snRNPs with the pre-mRNA 5' splice site and

branch site, respectively, generating the prespliceosome or complex A. This complex then recruits the pre-formed U4/U6.U5 tri-snRNP, in which the U4 and U6 snRNAs are base paired, to form the mature spliceosome or complex B. Conversion of the mature spliceosome into a machine capable of catalyzing splicing (hereafter termed the activated spliceosome) entails a major structural change that results in the dissociation of the U1 and U4 snRNPs. Subsequently, the activated spliceosome catalyzes the first transesterification step of splicing and complex C is formed. After the second step of splicing, the mRNA is released and the postsliceosomal complex, containing the excised intron and the U2, U5 and, U6 snRNPs, disassembles and the snRNPs are then recycled for new rounds of splicing.

The spliceosome is, thus, a highly dynamic molecular machine whose composition is not

¹Department of Cellular Biochemistry, Max Planck Institute of Biophysical Chemistry, D-37077 Göttingen, Germany. ²European Molecular Biology Laboratory, Bioanalytical Research Group, D-69117 Heidelberg, Germany.

*To whom correspondence should be addressed. E-mail: reinhard.luehrmann@mpi-bpc.mpg.de