mutants to determine that β-arrestin binds to a highly conserved cysteine-rich domain present in all diacylglycerol kinases, thus accounting for its interaction with all the enzyme isoforms. The authors also determined that diacylglycerol kinase ζ binds to a site in the C terminus of β-arrestin that is different from the one that binds to clathrin. What about other proteins that bind β-arrestin, including the small GTP-binding protein Rho and components of the MAP kinase signaling cascade, among others (*2*)? Only some of these binding sites have been mapped, and most appear distinct. Are arrestin dimers also required to enable receptor signaling regulation?

Nelson *et al*. also found that carbachol-stimulated phosphatidic acid production increased when β-arrestin was overexpressed in cells, whereas production decreased in cells depleted of β-arrestin—so low that a major role for β-arrestin in regulating phosphatidic acid production due to receptor activation is indisputable. In a related report, diacylglycerol kinase was isolated and localized with phosphatidylinositol 4-phosphate 5-kinase, an enzyme that is activated by phosphatidic acid and generates phosphatidylinositol 4,5-bisphosphate. Phosphatidylinositol 4,5-bisphosphate is the precursor for the second messenger diacylglycerol (*9*). It is unknown whether β-arrestin regulates the activity of phosphatidylinositol 4-phosphate 5-kinase directly or only through formation of phosphatidic acid.

The binding of β-arrestin to diacylglycerol kinases versus G protein–coupled receptors was discriminated by using a form of β-arrestin 2 lacking the N terminus, a region that his required for interaction with receptors.owever, this form could still bind to diacylglycerol kinases and acted as a dominant negative β-arrestin, causing retention of diacylglycerol kinase ζ in the cytosol. It also blocked phosphatidic acid production by the diacylglycerol kinase–β-arrestin pathway (rather than by phospholipase D). Because diacylglycerol is a second messenger for many diverse receptors, as well as a regulator of transient receptor potential channel activity, this role of β-arrestin may have widespread physiological consequences (*10*).

The findings by Nelson *et al*. are provocative because of the questions they raise: How much β-arrestin is required for a cell to regulate signaling normally? Is this different for distinct cells with discrete metabolic activity or modes of signaling? It is assumed that β-arrestin is present in all animal tissues and cells, but studies of β-arrestin localization are few. What other receptors types (*11*, *12*) are regulated in a similar manner? If β-arrestin similarly regulates signaling for other receptors, what other enzymes are recruited by β-arrestin? A whole new group of enzymes participating in signal termination may become the newest nonreceptor partner for β-arrestins (*2*, *7*).

References

1. E. Reiter, R. J. Lefkowitz, *Trends Endocrinol. Metab.* **17**, 159 (2006).
2. R. J. Lefkowitz, S. K. Shenoy, *Science* **308**, 512 (2005).
3. D. K. Morrison, R. J. Davis, *Annu. Rev. Cell Dev. Biol.* **19**, 91 (2003).
4. C. D. Nelson *et al*., *Science* **315**, 663 (2007).
5. S. J. Perry *et al*., *Science* **298**, 834 (2002).
6. V. V. Gurevich, E. V. Gurevich, *Pharmacol. Ther.* **110**, 465 (2006).
7. V. V. Gurevich, E. V. Gurevich, *Structure* **11**, 1037 (2003).
8. J. G. Krupnick, O. B. Goodman Jr., J. H. Keen, J. L. Benovic, *J. Biol. Chem.* **272**, 15011 (1997).
9. B. Luo, S. M. Prescott, M. K. Topham, *Cell Signal.* **16**, 891 (2004).
10. R. C. Hardie, *J. Physiol.* **578**, 9 (2007).
11. A. Puckerin *et al*., *J. Biol. Chem.* **281**, 31131 (2006).
12. F. T. Lin, Y. Daaka, R. J. Lefkowitz, *J. Biol. Chem.* **273**, 31640 (1998).

10.1126/science.1138505

ECOLOGY

Tackling Ecological Complexity in Climate Impact Research

Longer time scales and evaluation of temporal effects are essential for analysis of ecosystem perturbations.

Gian-Reto Walther

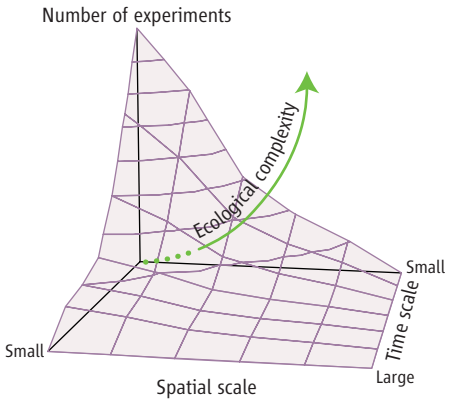
Evidence for the ecological impact of global climate warming has become increasingly compelling. For example, plants and animals adapt the timing of their life cycles or shift their ranges toward higher latitudes and/or altitudes in response to warmer climatic conditions (*1*). However, as the study by Suttle *et al.* (*2*) on page 640 of this issue shows, the observed responses of individual plant and animal species are just the starting point of a cascade of interweaved responses and feedback processes. In their field experiment, Suttle *et al.* imposed different projected precipitation regimes over grassland in California to evaluate the effects of these treatments on plant productivity and species composition of plants, invertebrate herbivores, and their natural enemies (*2*).

The author is in the Department of Plant Ecology, University of Bayreuth, 95440 Bayreuth, Germany. E-mail: gian-reto.walther@uni-bayreuth.de

The experiment stands out for several reasons. First, the 5-year time span of the experiment underlines the importance of experimental duration for drawing the right conclusions. The lifetime of experimental research rarely exceeds 2 to 3 years (*3*–*5*) (see the figure), although the manipulated system may need longer for the transition from initial disequilibrium to new equilibrium conditions. A field experiment in Arctic tussock tundra showed that focusing on individual species' responses over the short term (3 years) gave poor predictions of their long-term (9 years) response (*6*). Carbon dioxide (CO_2) enrichment experiments in established forest stands revealed that the stimulation of above-ground growth of forest trees in the first years was a temporary effect and was followed by a time-dependent (in this case 4 to 5 years) adjustment of the growth regime back to almost the same level as before CO_2 enrichment (*7*). Hence, the observed effects of manipulation depend heavily on the time period of data evaluation and suggest different interpretations.

In the grassland experiment by Suttle *et al*., the initial strong response of nitrogen-fixing forbs (that is, nongrass flowering plants with nonwoody stems) in the first few years was reversed through feedback processes, and the forbs were replaced by annual grasses, with consequences for both biodiversity and food web structure (*2*). Again, evaluation of the experiments after the first few years would have resulted in different trends and misleading inferences compared to those revealed in the longer run.

A second interesting feature of the Californian grassland experiment is how the projected precipitation regimes were implemented. Long-term mean precipitation is a useful indicator of the intensity of the hydrologic cycle, but the temporal and spatial patterns of the precipitation regime are the most important issues in determining impacts of



Not to scale. Ecological complexity increases with scale, whereas the number of ecological experiments shows the opposite trend. There is a strong tendency to ignore possible complicating factors of ecological complexity operating over a larger scale. This schematic chart is based on (*11*, *12*).

precipitation changes (*8*). In the grassland experiment, effects of increased rainfall depended strongly on the seasonality of the increase. Communities experiencing additional watering during the wet winter season responded similarly to those exposed to ambient rainfall, whereas watering at the end of the rainy season led to substantial and sustained changes in the composition of the affected communities (*2*). Hence, different temporal treatments representing the same trend of increases in annual precipitation, but with different seasonal patterns, highlighted the importance of the timing of rainfall.

There is a third reason for interest in these results. Although it is generally agreed that climate change will affect the temporal and spatial association between species interacting at different trophic levels, many studies concentrate on the effects of a single variable on a particular species. Thus, there is a strong tendency to ignore possible complicating factors operating over larger scales and/or multitrophic levels (*4*, *9*). Long-term field data from grassland habitats in Germany indicate that different trophic levels respond differently to climate fluctuations and suggest that differential trophic responses are likely to be a common, widespread, and important phenomenon (*10*). The community-level interactions considered in the experiment by Suttle *et al*. were strongly influenced by persistent altered environmental conditions and not a consequence of short-term fluctuations of the precipitation regime. The initial signal of increased plant species richness and greater diversity and abundance of invertebrate herbivores, predators, and parasitoids was reversed in the course of the experiment as a result of the increasing share of annual grasses with low nutritional value and monocultures of these plants offering low structural complexity. This finally led to a simplification of the grassland community as a whole (*2*).

Together, these examples warn against overstating the results of inappropriate experimental conditions in terms of temporal [and also spatial (*4*, *11*)] scales (see the figure). Careful interpretation of experimental data is thus crucial to avoid overinterpretation of early experimental results. In general, time-series analysis should be performed over as many years as possible.

The study by Suttle *et al.* shows how one can tackle ecological complexity in manipulative experiments: The 5-year duration of the experiment has revealed reversal trends of initial effects; different temporal patterns of treatment highlight the importance of the timing of rainfall compared to the annual amount; and feedback processes through higher trophic levels may overturn direct climatic effects on the species level and reverse community trajectories. These aspects make this work particularly valuable for ecological experiments and for global change research in general. It is a strong reference for the importance of field-based long-term monitoring and experiments for climate impact studies.

References and Notes

1. C. Parmesan, *Annu. Rev. Ecol. Evol. Syst.* **37**, 637 (2006).
2. K. B. Suttle, M. A. Thomsen, M. E. Power, *Science* **315**, 640 (2007).
3. G. E. Likens, Ed., *Long-Term Studies in Ecology* (Springer, New York, 1989).
4. P. J. Edwards, R. M. May, N. R. Webb, Eds., *Large-Scale Ecology and Conservation Biology* (Blackwell Scientific Publ., Oxford, UK, 1994).
5. S. J. Andelman, M. R. Willig, *Science* **305**, 1565 (2004).
6. F. S. Chapin III, G. R. Shaver, A. E. Giblin, K. J. Nadelhoffer, J. A. Laundre, *Ecology* **76**, 694 (1995).
7. Ch. Körner, *New Phytol.* **172**, 393 (2006)
8. M. R. Allen, W. J. Ingram, *Nature* **419**, 224 (2002).
9. R. Harrington, I. Woiwood, T. Sparks, *Trends Ecol. Evol.* **14**, 146 (1999).
10. W. Voigt *et al*., *Ecology* **84**, 2444 (2003).
11. P. Kareiva, M. Andersen, in *Community Ecology*, A. Hastings, Ed. (Springer-Verlag, New York, 1989), pp. 35–50.
12. D. Tilman, in *Long-term Studies in Ecology*, G. E. Likens, Ed. (Springer, New York, NY, 1989), pp. 136–157.
13. I acknowledge funding by the European Union within the FP6 Integrated Project "ALARM" (GOCE-CT-2003-506675) and by the German Federal Agency of Nature Conservation (FKZ 805 81 001).

10.1126/science.1138574

CELL SIGNALING

Mitochondrial Longevity Pathways

György Hajnóczky and Jan B. Hoek

A cytosolic protein that translocates into the mitochondria may serve as an integration point for signaling pathways that control longevity and cell death.

The quest for longevity has led to the discovery of several genes that affect the life span of organisms ranging from yeast to mammals. An increased life span has been linked to the expression of sirtuins, impaired function insulin receptor homologs, and absence of the signaling protein $p66^{Shc}$. Several cell signaling pathways associated with these factors converge on the Forkhead/FOXO family of transcription factors, which regulate the expression of a battery of stress response proteins that affect antioxidant capacity, cell cycle arrest, DNA repair, and apoptosis (*1*). Life span–regulating proteins also directly affect mitochondrial function, including energy metabolism and reactive oxygen species production, in which $p66^{Shc}$ plays a critical role. How these mitochondrial processes integrate with the upstream signaling events to control life span has remained enigmatic. On page 659 of this issue, Pinton *et al*. describe a signaling pathway that controls the mitochondrial activity of $p66^{Shc}$ (*2*) (see the figure) and provides insight into how this integration might occur.

The extended life span of mice lacking $p66^{Shc}$ has been correlated with a decrease in mitochondrial metabolism (*3*) and reactive oxygen species production (*4*). Pinton *et al*. show that $p66^{Shc}$ is required for early mitochondrial responses to an oxidative challenge (hydrogen peroxide, H_2O_2). These responses include mitochondrial fragmentation and suppression of Ca^{2+} signal propagation to the mitochondria, followed by execution of apoptosis (cell death) in murine fibroblasts. The authors found that early mitochondrial response to H_2O_2 increased progressively with cell culture age, and used this model to map the signaling cascade through which $p66^{Shc}$ affects mitochondria.

The authors are in the Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107, USA. E-mail: gyorgy.hajnoczky@jefferson.edu, jan.hoek@jefferson.edu

Science
AAAS

Tackling Ecological Complexity in Climate Impact Research

Gian-Reto Walther

Science **315**, 606 (2007);
DOI: 10.1126/science.1138574