is also induced at the G_1-S transition in non–heat-shocked cells (*11*). Our results raise the possibility that the c-Myb–induced activation of HSF3 contributes to the cell cycle–dependent expression of stress-responsive genes.

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23. The preparation of GST-HSF fusion proteins, in vitro translation of c-Myb, and binding assays were as described (*19*) except for the binding buffer used [20 mM Hepes (pH 8.5), 1 mM EDTA, 5 mM dithiothreitol, and 0.1% NP-40], which contained 70 or 150 mM NaCl (for Fig. 3A).
24. We constructed the plasmid to express the Gal4-HSF fusion proteins containing the Gal4 DNA binding domain (amino acids 1 to 147) joined to either the DNA binding domain of HSF1 (amino acids 1 to 132), HSF2 (amino acids 1 to 130), or HSF3 (amino acids 1 to 125), by the polymerase chain reaction–based method with the use of the cytomegalovirus promoter–containing expression vector. The plasmid to express the Myb-VP16 fusion protein containing the DNA binding domain of c-Myb (amino acids 1 to 193) joined to the VP16 activation domain was made similarly with the use of the pcDNA3 vector (Invitrogen). In the reporter plasmid, the thymidine kinase promoter containing three copies of the Gal4-binding site was linked to the luciferase gene. A mixture containing 4 μg of the luciferase reporter plasmid, 2 μg of the Gal4-HSF expression plasmid, 8 μg of either the VP16-Myb or VP16 expression plasmid, and 1 μg of pact-β-gal was transfected into NIH 3T3 cells, and luciferase assays were performed. Total plasmid DNA was adjusted to 17 μg by the addition of pact-1 DNA.
25. The 293T cells were transfected with either 0.3 μg of the chicken HSF3 expression plasmid or 8 μg of the mouse c-Myb expression plasmid, or a mixture of both plasmids. The transfected DNA also contained 0.5 μg of pact–β-gal DNA (*10*), and the total amount of plasmid DNA was adjusted to 10 μg by the addition of pact-1 DNA (*10*). Immunostaining and protein immunoblotting of HSF3 were performed as described (*8*).

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Influence of Food Web Structure on Carbon Exchange Between Lakes and the Atmosphere

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Top predators and nutrient loading in lakes were manipulated to assess the influence of food web structure on carbon flux between lakes and the atmosphere. Nutrient enrichment increased primary production, causing lakes to become net sinks for atmospheric carbon (C_{atm}). Changes in top predators caused shifts in grazers. At identical nutrient loading, C_{atm} invasion was greater to a lake with low grazing than to one with high grazing. Carbon stable-isotope distributions corroborated the drawdown of lake carbon dioxide and traced C_{atm} transfer from algae to top predators. Thus, top predators altered ecosystem carbon fixation and linkages to the atmosphere.

In many lakes, carbon (C) inputs from terrestrial systems are sufficiently high that lakes are supersaturated with CO_2, and there is net diffusion of CO_2 out of surface water, making lakes conduits of C from the terrestrial environment to the atmosphere (*1–3*). In productive lakes, primary production by algae and C storage in biota and sediments are high, so that aqueous CO_2 is depleted and C_{atm} diffuses into surface waters (*2*, *4*).

Primary production by algae in lakes is determined by interactions among a variety of factors that include nutrient loading (*5*) and food web structure (*6*). In general, primary production is high in lakes with large nutrient loads. Food web structure is often determined by the feeding characteristics of fishes. Lakes with planktivorous fishes are generally characterized by small bodied zooplankton grazers (*7*) that are less effective at suppressing algal abundance and growth than are communities dominated by large bodied grazers that often coexist with piscivorous fishes (*6*). Thus, food web structure as determined by the dominant feeding modes of predatory fishes has the potential to regulate aquatic primary production and C fluxes between lakes and the atmosphere.

We independently manipulated fish communities and nutrient loading rates in four lakes to test the interactive effects of nutrient loading and food web structure on lake productivity and C exchange with the atmosphere (Table 1). Two lakes were dominated by zooplanktivorous fishes (minnows), and two by piscivores (bass) (*8*). From 1993 to 1995, one lake from each food web configuration was enriched with nitrogen and phosphorus, and the two other (reference) lakes were monitored without fertilization (Table 1).

At ambient nutrient loads, mean summer primary production rates by pelagic algae ranged from 170 to 414 mg C·

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$m^{-2} \cdot day^{-1}$ among all lakes (Fig. 1A). Nutrient enrichment increased primary production in both the piscivore lake and the planktivore lake. However, at enhanced nutrient loads, the planktivore lake always had higher primary production than the piscivore lake. At equivalent nutrient loads, the mean summer primary production rates in the planktivore lake were 1.4 to 2.9 times higher than in the piscivore lake (Fig. 1A).

Before nutrient enrichment, all lakes were net sources of C to the atmosphere, and C efflux rates ranged from 24 to 113 mg $C \cdot m^{-2} \cdot day^{-1}$ (Fig. 1B). After nutrient enrichment, C efflux decreased with increases in primary production such that enriched lakes were either approximately in equilibrium with atmospheric CO_2 or were net sinks for atmospheric carbon (C_{atm}). Summer mean influx of C_{atm} at enhanced nutrient loads ranged from 101 to 140 mg $C \cdot m^{-2} \cdot day^{-1}$ to the planktivore lake and from 0 to 30 mg $C \cdot m^{-2} \cdot day^{-1}$ to the piscivore lake. These differences in C_{atm} influx rates to lakes are due to regulation of primary production by food web structure. In the piscivore-dominated lake, planktivorous fishes were essentially eliminated, and a large-bodied herbivorous zooplankton community dominated by *Daphnia pulex* was present (9). Because these large grazers are able to suppress algal growth responses to nutrient enrichment (6, 9, *10*), the effect of increased nutrient load on primary production is weak in piscivore-dominated lakes. In planktivore-dominated lakes, algae are not suppressed by grazers, and primary production is sensitive to increases in nutrient load (9). Thus, algae are more effective at depleting dissolved CO_2 in planktivore-dominated lakes and allow C_{atm} to diffuse down the concentration gradient from the atmosphere into surface waters.

Cole *et al.* (3) reported that the global variation in lake CO_2 concentration ranges from about 16 times undersaturated to about 16 times supersaturated. We observed most of this range in relative CO_2 concentration in our experiment. The fertilized zooplanktivore lake was about 16 times undersaturated with CO_2, unfertilized lakes were about four times supersaturated with CO_2, and the fertilized piscivore lake was close to equilibrium with the atmosphere. Thus, the potential magnitude of the effects of food web structure on C exchange between lakes and the atmosphere is substantial.

A brief perturbation to the food web of Peter Lake confirmed that food web dynamics can produce the shifts in primary production and dissolved CO_2 observed between experimental lakes (Fig. 2). The mean length of zooplankton grazers ranged from 0.2 to 1.0 mm in 1993 to 1995 as a result of a partial minnow die-off in 1994 and subsequent population recovery in 1995 (9, *11*).

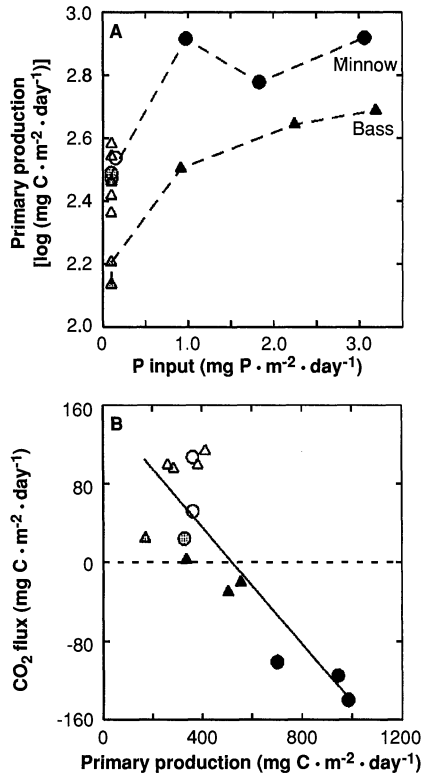

Fig. 1. (**A**) Effect of P input rate on primary production in four lakes with contrasting food web configurations. Each symbol represents a summer mean for one lake*year combination from 1991 to 1995. Lakes characterized by high planktivory and low grazing rates are shown by circles. Triangles represent lakes with piscivores and high grazing rates. Lakes that were experimentally fertilized are denoted by filled symbols: gray symbols are lake*years before enrichment, and black symbols are during enrichment. (**B**) Relation between calculated CO_2 flux between lakes and overlying atmosphere (*18*), and the estimated primary production rate in 1992 to 1995 (correlation −0.84, $P < 0.001$). Positive values of CO_2 flux represent net flow of C out of the lake, and negative values represent net flow of C into the lake. The dashed line represents lakes that are in equilibrium with the overlying atmosphere with respect to CO_2 concentration. CO_2 flux rates were only measured in 1994 and 1995 for Tuesday Lake (open circles). Symbols are as described in (A).

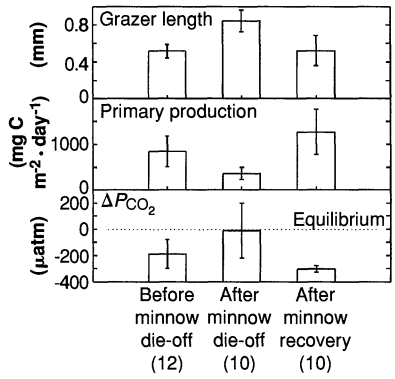

Fig. 2. Comparison of crustacean grazer length, primary production, and ΔP_{CO_2} [$P_{CO_{2(lake)}} - P_{CO_{2(air)}}$] before, during, and after a period of low minnow abundance in Peter Lake (1994 to 1995). Data are shown as the mean ± SD. Sample sizes are given in parentheses. Dotted line in bottom panel represents dissolved CO_2 in equilibrium with the atmosphere.

Table 1. Lake characteristics and design of whole lake experiments to test the interacting effects of nutrient loading and food web structure on primary production rates and C exchange between lakes and the atmosphere. All lakes are located at the University of Notre Dame Environmental Research Center near Land O' Lakes, Wisconsin (89°32′W, 46°13′N). Values of water transparency (Secchi depth), depth of the mixed layer, and mixed layer dissolved inorganic carbon (DIC) are long-term means taken from weekly samples during the stratified season from 1991 to 1995. The food web structures of Peter Lake and Tuesday Lake were manipulated in May 1991 and September 1991, respectively, by rotenone treatment to remove all piscivores, followed by restocking of planktivorous species. Liquid fertilizer containing PO_4, NH_4, and NO_3 at an N:P atomic ratio of 25 was added from a central station in Peter Lake and West Long Lake from May to September in 1993, 1994, and 1995 (9). P loading rates for these 3 years were 3.06, 1.83, and 0.97 mg $P \cdot m^{-2} \cdot day^{-1}$ for Peter Lake, and 3.19, 2.24, and 0.92 mg $P \cdot m^{-2} \cdot day^{-1}$ for West Long Lake. Baseline P loading rates are about 0.1 to 0.15 mg $P \cdot m^{-2} \cdot day^{-1}$. Primary production was measured biweekly, and CO_2 fluxes weekly, from 1991 to 1995 in all lakes except Tuesday Lake. In Tuesday Lake, primary production was measured in 1989 when the lake was planktivore-dominated and not enriched with nutrients (6). Planktonic primary production was measured at six depths by the ^{14}C method. Daily integrated C fixation was calculated by continuous measurements of surface irradiance and weekly profiles of light attenuation, temperature, chlorophyll, and dissolved inorganic C (6). C stable-isotope distributions in the pelagic food webs were monitored in 1994 and 1995. Lake characteristics are described in detail in (6).

Lake	Area (ha)	Mean depth (m)	Secchi depth (m)	Mixed layer depth (m)	Mixed layer DIC (mg C liter^{-1})	Nutrient status
			Piscivores, large herbivores			
Paul Lake	1.7	3.7	4.6	3.6	1.6	Ambient
West Long Lake	3.4	4.7	3.4	3.5	0.6	Enriched
			Zooplanktivores, small herbivores			
Tuesday Lake	1.2	6.9	2.7	2.8	0.8	Ambient
Peter Lake	2.7	5.7	3.9	3.7	1.6	Enriched

In correspondence with the food web changes, the partial pressure of CO_2 (P_{CO_2}) ranged from approximately in equilibrium with the atmosphere to greatly undersaturated relative to the atmosphere (*12*).

We used C stable isotope distributions in the biota to confirm in-lake CO_2 drawdown and trace C_{atm} through the pelagic food webs of the experimental lakes (*13*). C_{atm} exhibits high ^{13}C:^{12}C ratios relative to aqueous CO_2 produced from terrigenous C (*14*, *15*). This enriched isotopic composition of C_{atm} translates into a relatively high $\delta^{13}C$ in algae when their growth is supported by fixation of C_{atm} (*15*). Drawdown of lake CO_2 concentration also changes the C stable isotope distribution because algal fractionation of C isotopes is reduced when CO_2 is diminished from water (*16*).

When net C flux was out of the lakes—that is, at ambient nutrient loads—C stable isotope distributions ($\delta^{13}C$) of planktonic algae, zooplankton, and fishes were between −30 and −36‰ (Fig. 3), indicating the importance of terrigenous C in supporting growth of aquatic biota (*15*). Enhancing primary production through nutrient enrichment caused substantial decreases in CO_2 fugacity that resulted in changes in the rates and direction of C flux between lakes and the atmosphere. The increased contribution of C_{atm} in food webs is illustrated by higher values of $\delta^{13}C$ in algae, zooplankton, and fishes. The greatest proportion of C_{atm} was observed in the planktivore-dominated lake (Fig. 3), where grazers were unable to suppress primary production rates with nutrient enrichment (Fig. 1A). These results demonstrate that top predators can influence C flow between ecosystems and the atmosphere through their effects on primary producers. This finding elaborates upon earlier discoveries that nutrient enrichment increases algal productivity and decreases CO_2 fugacity in lake (*2*, *4*) and open ocean (*17*) ecosystems.

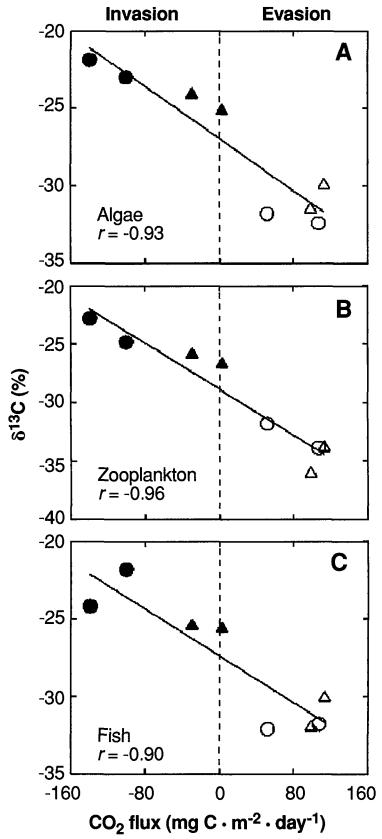

Fig. 3. Carbon stable isotope distributions of three pelagic food web components [(**A**) Algae, (**B**) zooplankton, and (**C**) fish] as a function of CO_2 flux rate between lakes and the atmosphere (correlation coefficients shown, all $P < 0.001$). Invasion of C_{atm} into lakes due to CO_2 drawdown results in relatively high values of $\delta^{13}C$. Lower values represent decreasing degree of CO_2 drawdown and an increasing importance of terrigenous C in biota. Data are shown for 1994 and 1995 for each of the four lakes in the experiment. Negative values of CO_2 flux represent net flow of C into lakes, and positive values represent net flow out of lakes. Symbols are as described in Fig. 1.

By independently manipulating food web structure and nutrient loading at the scale of whole ecosystems, we demonstrated that top predators alter fundamental biogeochemical processes that control internal ecosystem dynamics and interactions with the atmosphere. Shifts in top predators determined whether the experimentally enriched lakes operated as net sinks or net sources of C_{atm}.

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8. Paul Lake had largemouth bass (*Micropterus salmoides*) as a top predator; West Long Lake had largemouth bass and smallmouth bass (*M. dolomieu*). The two planktivore-dominated lakes (Tuesday Lake and Peter Lake) had mixed assemblages composed of fathead minnows (*Pimephalus promelas*), redbelly dace (*Phoxinus eos*), and golden shiners (*Notemigonus crysoleucas*).
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11. Biomass of minnows in Peter Lake declined from 9 g wet mass per square meter to less than 2 g wet mass per square meter after a partial die-off in late July 1994. Populations remained at low densities until successful spawning in June 1995. By mid-August 1995, minnow biomass had recovered to 10 g wet mass per square meter. Variability in population densities of planktivorous fishes is often due to density-dependent interactions and is relatively common in lakes [S. R. Carpenter and P. R. Leavitt, *Ecology* **72**, 277 (1991); S. F. Hamrin and L. Persson, *Oikos* **47**, 223 (1986)].
12. To evaluate the effect of crustacean zooplankton grazer length (X_t) on primary production rate and degree of CO_2 saturation, we used least-squares regression to fit the following time series model [G. E. P. Box, G. M. Jenkins, G. C. Reinsel, *Time Series Analysis: Forecasting and Control* (Prentice-Hall, Englewood Cliffs, NJ, 1994)] to data collected weekly from Peter Lake in 1993 to 1995:

$$Y_t = B_o + \phi Y_{t-1} + B_1 X_t + \varepsilon_t$$

Y_t is the response variable at time t, B_o is a constant, ϕ is an autoregressive parameter that corrects for serial dependency in the time series of Y_t, and B_1 describes the effect of X_t on Y_t. We detected a significant, negative effect of grazer length on log (primary production rate) ($B_1 = -0.461$, SE = 0.175, $t = -2.633$, $P = 0.012$). This model also included a positive constant ($B_o = 1.670$, SE = 0.415, $t = 4.022$, $P = 0.000$) and a lag-1 autoregressive term ($\phi = 0.512$, SE = 0.126, $t = 4.054$, $P = 0.000$). To evaluate changes in the degree of CO_2 saturation that paralleled changes in grazer size, we calculated the difference (Δ) between P_{CO_2} in the surface water and the P_{CO_2} of the overlying atmosphere. Negative values of Δ represent undersaturated conditions, and positive values represent supersaturated conditions. We transformed Δ to normalize residuals according to log ($\Delta + \Delta_{min+1}$), where Δ_{min+1} is a constant (404) to make the series positive. We detected a significant, positive effect of grazer length on Δ in Peter Lake ($B_1 = 0.451$, SE = 0.209, $t = 2.160$, $P = 0.037$). This model also included a positive constant ($B_o = 1.453$, SE = 0.193, $t = 7.517$, $P = 0.000$) and a lag-1 autoregressive term ($\phi = 0.227$, SE = 0.088, $t = 2.587$, $P = 0.013$). Residuals of both time series models had no significant autocorrelations and were approximately normally distributed.
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tope standard (PeeDee belemnite) according to

$\delta^{13}C = [(^{13}C/^{12}C_{sample} \div {}^{13}C/^{12}C_{standard}) - 1] \times 1000$

Isotope ratios were determined with a Europa 20-20 continuous-flow mass spectrometer with reproducibility of 0.2 per mil.

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18. The partial pressure of CO_2 (P_{CO_2}) was obtained from direct measurement in the surface water (0.005 to 0.01 m depth) of each lake by a headspace-equilibrium technique in a specially designed 2-liter bottle (*3*). At the same time, samples were taken of CO_2 in the overlying atmosphere, 1 m above the lake. Gas chromatography was used to measure the extracted gas. The aqueous concentration of CO_2 [$CO_{2(water)}$] was then calculated from temperature and temperature-corrected values of K_h (Henry's constant) by use of the relations in R. F. Weiss [*Mar. Chem.* **2**, 203 (1974)]. To estimate flux, we used the equation $CO_2 = \propto k[CO_{2(water)} - CO_{2(sat)}]$, where $CO_{2(sat)}$ is the concentration the water would have were it in equilibrium with the atmosphere, and k is the gas-exchange coefficient expressed as a piston velocity. For these low wind lakes we assumed a constant value for k of 2 cm · hour^{-1}. $\propto$ is the chemical enhancement factor. When the formulation in Hoover and Berkshire was used [T. E. Hoover and P. C. Berkshire, *J. Geophys. Res.* **74**, 456 (1969)], $\propto$ was significantly different from 1.0 only in Peter Lake (undersaturated, high pH) and averaged about 3.0.
19. We thank K. Cottingham, J. Hodgson, D. Thomas, and N. Voichick for assistance with experiments and sampling; S. Blumenshine, N. Caraco, K. Cottingham, T. Kratz, D. Lodge, Y. Vadeboncoeur, and K. Webster for helpful discussions; and T. Frost and R. Hellenthal for logistical support. N. Haubenstock and K. Tholke performed the stable isotope analyses. This manuscript benefited from reviews by P. Leavitt and K. Webster. Financial support was provided by the National Science Foundation–Ecosystems Program and Doctoral Dissertation Improvement Program. This is a contribution to the University of Wisconsin, Center for Limnology, the Institute of Ecosystem Studies, and the University of Notre Dame Environmental Research Center.

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Dictyostelium Development in the Absence of cAMP

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Adenosine 3′,5′-monophosphate (cAMP) and cAMP-dependent protein kinase (PKA) are regulators of development in many organisms. *Dictyostelium* uses cAMP as an extracellular chemoattractant and as an intracellular signal for differentiation. Cells that are mutant in adenylyl cyclase do not develop. Moderate expression of the catalytic subunit of PKA in adenylyl cyclase–null cells led to near-normal development without detectable accumulation of cAMP. These results suggest that all intracellular cAMP signaling is effected through PKA and that signals other than extracellular cAMP coordinate morphogenesis in *Dictyostelium*.

In both vertebrates and invertebrates, the control of cAMP synthesis and its detection by cellular targets such as PKA are essential for many developmental processes (*1*). During *Dictyostelium* development, cAMP is used as a signal both inside and outside of cells (*2*). Pulses of extracellular cAMP are generated by the cells and are used for chemotaxis during aggregation, a process that brings 10^5 cells into a mound. Extracellular cAMP is detected by G protein–coupled cell surface receptors (*3*). This results in the chemotaxis of cells toward increased cAMP as well as propagation of the signal through the activation of ACA, the adenylyl cyclase that produces cAMP during development (*4*). PKA, the major downstream effector of the cAMP-ACA signaling pathway inside the cell, is activated when its regulatory subunit (PKA-R) binds cAMP and dissociates from the catalytic subunit (PKA-C) (*5*). Cells in which PKA-R is inactivated (*6*) or in which PKA-C is constitutively active (*7*, *8*) develop rapidly, which suggests that PKA regulates the timing of development.

After aggregation, extracellular cAMP has been proposed to direct a number of processes, including the sorting of cell types into distinct tissues, slug migration, terminal cell differentiation, and the morphogenesis of the final fruiting body (*9*–*11*). To test the role of extracellular cAMP in development, we obviated the requirement for cAMP as a second messenger by rendering PKA constitutively active. By doing this in an ACA-null mutant, we produced cells that do not make cAMP but have the key intracellular cAMP pathway activated. Because PKA activation promotes many developmental events (*7*, *8*, *11*, *12*), these cells allowed an assessment of the processes that are independent of cAMP.

We introduced an expression plasmid with an epitope-tagged PKA-C coding region under the transcriptional control of an *actin15* promoter into *acaA* mutant cells by transformation (*13*). The *actin15* promoter directs relatively constant amounts of transcription throughout development in all cells (*14*). After the primary transformants were cloned on growth plates, cells that had exhausted the bacterial food supply within some of the colonies were clearly undergoing development. The epitope-tagged PKA-C protein was produced by these developing transformants [*acaA*(PKA-C) cells] but was not detected in transformants that did not develop (*13*). Analysis of the DNA and RNA of several isolates of *acaA*(PKA-C) cells confirmed that the *acaA* locus remained disrupted and that *acaA* mRNA did not accumulate at any time during development (*13*). We also measured PKA-C activity in cells that had been developing for 8 hours, as measured by phosphorylation of the peptide substrate Kemptide (*15*). The *acaA*(PKA-C) cells exhibited 4.3 times the activity (1.4 nmol min^{-1} mg^{-1}) of the parental *acaA* cells and 1.6 times the activity of wild-type cells.

When *acaA*(PKA-C) cells were axenically grown, washed, and placed on Millipore filters, they underwent relatively normal development. They began to aggregate by 10 hours, forming broad "streams" of cells that persisted for several hours, such that mound formation was delayed by about 2 hours (Fig. 1A). The mounds produced by the *acaA*(PKA-C) cells progressed through a normal sequence of developmental structures and formed fruiting bodies by 30 hours (Fig. 1, B and C).

To determine whether constitutive PKA activity restored cAMP signaling in the *acaA*(PKA-C) cells, we measured adenylyl cyclase activity and cAMP production in several ways. After cells were pulsed with cAMP for 5 hours, *acaA*(PKA-C) cells had <0.4% of the adenylyl cyclase activity of wild-type cells (*16*). During development on filters, adenylyl cyclase activity in wild-type cells increased to a maximum at 8 hours and then declined, as previously reported (*17*). The activity in *acaA*(PKA-C) cells and *acaA* mutant cells never rose above the background of the assay (*16*). We also directly measured the accumulation of cAMP in intact aggregation-competent cells (*18*). When cells were stimulated with 2′-deoxy-cAMP, cAMP rapidly accumulated in wild-type cells but remained at background in the *acaA*$^-$ and *acaA*(PKA-C) cells (Fig. 2A). During the development of

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