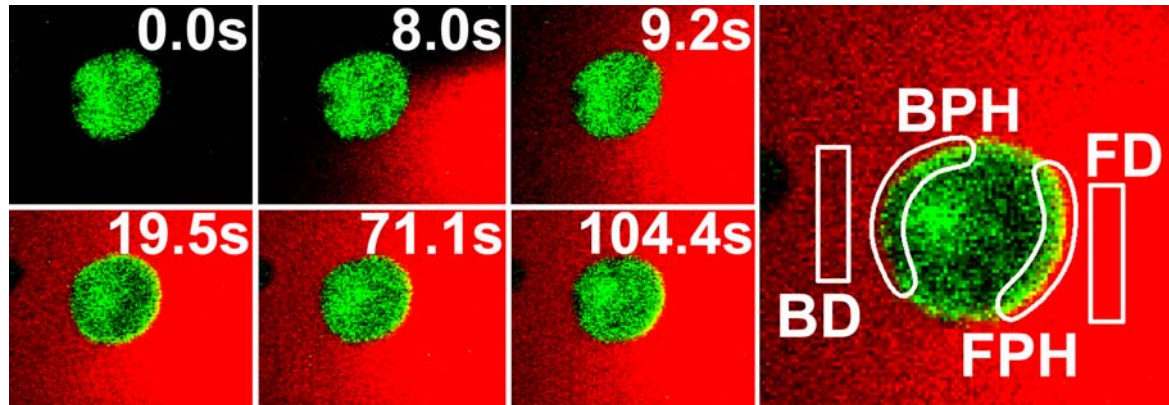
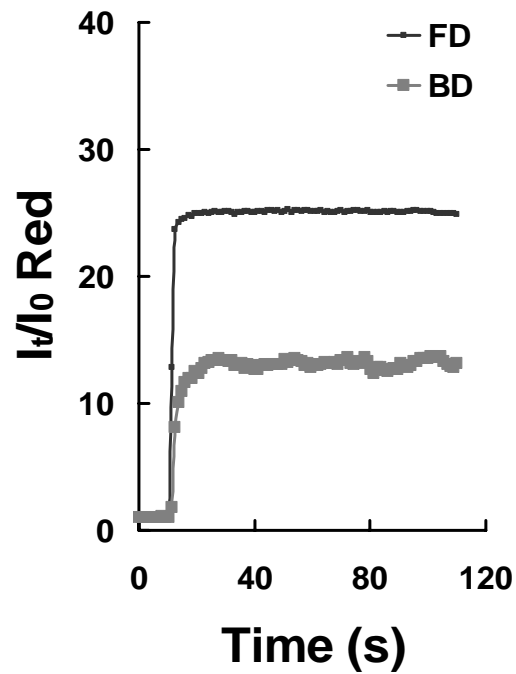


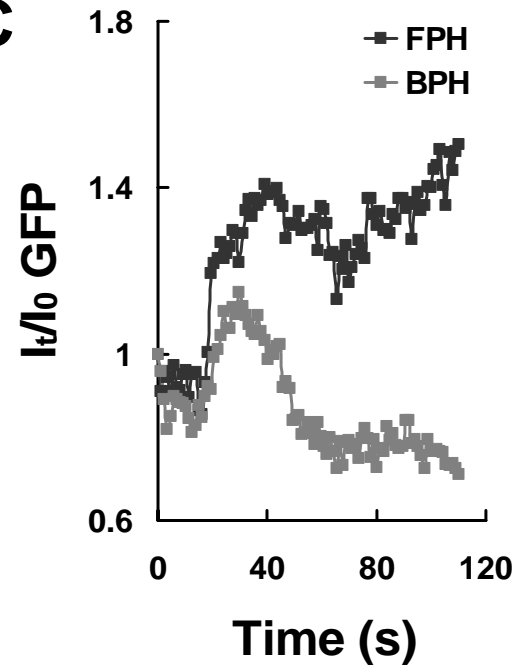
A



B



C



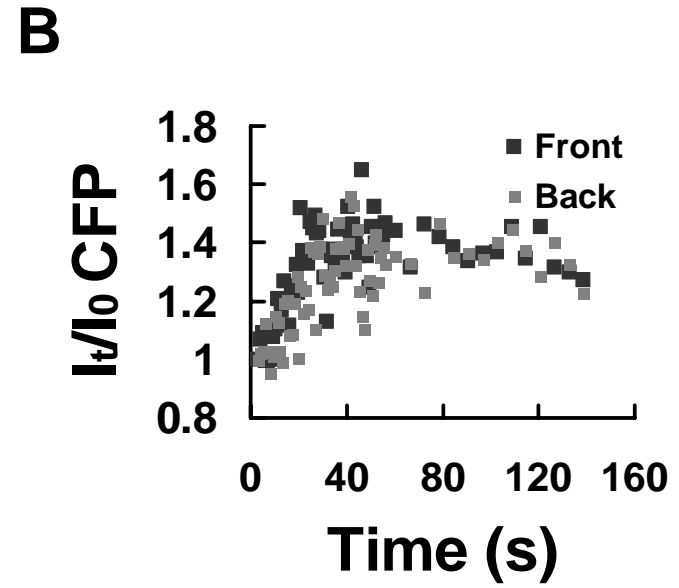
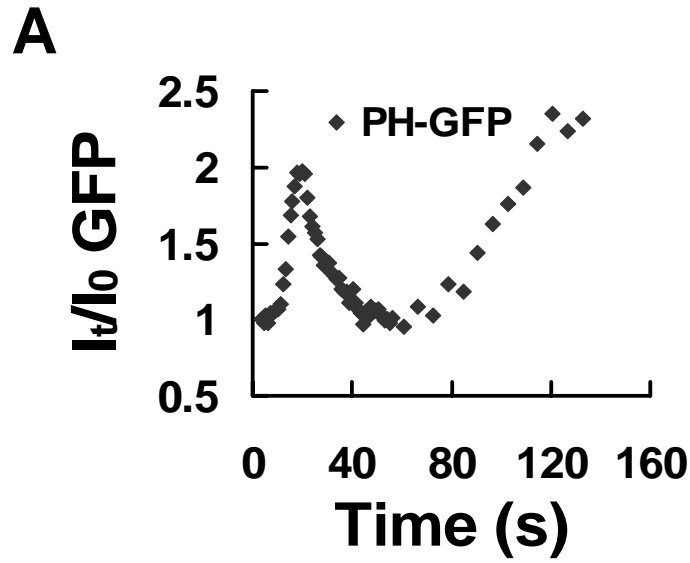


Table 1. cAMP-dose-dependent T_{\max} and R_{\max} of transient PH_{Crac} -GFP response in a uniform field of cAMP.

cAMP induces a transient membrane translocation of PH_{Crac} -GFP from the cytosol. As shown in Fig. S1, temporal changes in the amount of cytosolic PH_{Crac} -GFP were measured and normalized, and T_{\max} and R_{\max} were determined for individual cells, as shown below. Means and SD for each concentration were calculated, and are listed below and shown in Fig. 3A and 3B.

100 nM	T_{\max} (s)	R_{\max}	10 nM	T_{\max} (s)	R_{\max}	1 nM	T_{\max} (s)	R_{\max}
1	7.18	0.742251	1	11.87	0.616195	1	12.92	0.610976
2	6.63	0.809759	2	10.62	0.522209	2	14.28	0.795412
3	7.18	0.738178	3	9.37	0.443503	3	13.6	0.730963
4	9.14	0.689611	4	9.37	0.571927	4	12.92	0.768168
5	8.49	0.725649	5	10	0.717982	5	14.28	0.646853
6	7.84	0.716701	6	8.75	0.620189	6	12.92	0.773035
7	7.18	0.782763	7	15.41	0.486842	7	13.6	0.821126
8	10.45	0.694245	8	16.64	0.505875	8	10.46	0.843822
9	4.86	0.610934	9	14.17	0.448624	9	10.46	0.690028
10	4.86	0.479146	10	13.56	0.472686	10	10.46	0.750927
11	4.86	0.690384	11	13.56	0.483301	11	9.59	0.80457
12	6.08	0.754848	12	11.71	0.623408	12	10.46	0.841153
13	6.08	0.535478	13	9.59	0.723928	13	11.04	0.57083
14	4.35	0.613966	14	8.85	0.699673	14	10.25	0.531828
15	4.35	0.674688	15	8.85	0.681977	15	10.25	0.577943
16	6.08	0.585849	16	10.32	0.732493	16	9.46	0.490334
17	6.8	0.551714	17	9.59	0.724613	17	11.04	0.573259
18	8.5	0.582319	18	10.24	0.586385	18	11.04	0.570115
19	7.65	0.62531	19	8.04	0.594666	19	11.04	0.579336
20	9.35	0.512887	20	8.77	0.820632	20	11.04	0.572386
21	7.65	0.600273	21	7.31	0.851704	21	8.88	0.682623
22	7.65	0.600273	22	9.51	0.628353	22	9.68	0.737232
23	7.65	0.724033	23	9.51	0.67268	23	8.88	0.749522
24	6.8	0.624862	24	7.31	0.745667	24	9.68	0.738248
25	7.65	0.659453	25	8.04	0.7265			
26	7.65	0.681144	26	8.04	0.693522			
27	6.8	0.728815	27	8.04	0.633352			
28	6.8	0.67465	28	6.58	0.797532			
29	7.65	0.727049						

Mean	T_{\max} (s)	SD	Mean	R_{\max}	SD
100nM	7.04	1.47	100nM	0.659904	0.082655
10nM	10.13	2.5	10nM	0.636658	0.114306
1nM	11.2	1.67	1nM	0.685445	0.10791

FigS1. Concentration-dependent transient responses of PH_{Crac}-GFP in uniformly applied cAMP stimulations.

(A) Relative intensity of PH_{Crac}-GFP transiently decreases in cytosol of a cell upon a uniform stimulation. Responses to 100 nM (3 cells) and to 10 nM (6 cells) are shown. PH_{Crac}-GFP expressing cells were differentiated for 5 hrs and treated with Latrunculin before being stimulated with cAMP. Translocation responses were normalized as the ratio of the mean GFP intensity at any given time (I_t) to that of time 0 (I_0) when cAMP was added. The lowest point indicates the value of T_{max} and R_{max} for each cell response; these values are shown in Table 1. (B) G-protein activation shown as the CFP intensity changes on the membrane in one cell stimulated with a low dose (1nM, gray) and then a high dose (100 nM, black). (C) PH_{Crac}-GFP response in one cell stimulated with a low dose (1nM, gray) and then a high dose (100 nM, black).

FigS2. Quantitatively measuring gradients of extracellular cAMP.

(A) An image of a circular cAMP gradient. A microinjector was linked to a Femtojet with constant pressure ($P_c=70$ and $P_i=70$), which injects a constant and small volume of mixture of Alexa 594 and cAMP solution into a one-well cell chamber with total buffer volume of 6 ml. When the micropipette is moved from one position to another, a gradient remains almost constant (Video9.mov.). (B) A normalized intensity change of red fluorescence along the thick green line in FigS2.A shows an exponential decay curve as a function of the distance (μm). The steepness of the gradient is defined as, $(I_f - I_b)/I_b$, where I_f is the intensity at the front of a cell and I_b is the intensity at the back. A cell is about 10 μm in diameter, therefore, the gradient across a cell residing in the range from 30 to 80 μm to the position of a micropipette is estimated at about 20%. (C). Images of a circular gradient of a mixture of two fluorescence dyes that differ to a similar degree in their molecular masses as do cAMP (MW 329.2) and Alexa594 (MW 758.79). A micropipette filled with a mixture of LysoTracker Red DND-99 (MW 399.025, red) and Alexa fluor 488 (MW 643.41, green) generated a stable gradient. Fluorescence images of LysoTracker Red DND-99 (red) or Alexa fluor 488 (green) of the gradient were recorded simultaneously in two channels. Channel one: excitation is 543 nm and emissions from 585-615 nm to specifically monitor LysoTracker Red DND-99; Channel two: excitation

is 488 nm and emissions from 505-530 to specifically monitor Alexa fluor 488. (D) A comparison a comparison of the diffusion concentration profiles for two dyes, LysoTracker Red DND-99 and Alexa fluor 488,. Quantitative measurement of the gradient along the line starting from the position of the dispensing micropipette are shown as intensities of LysoTracker Red DND-99 (red) and Alxea fluor 488 (green) as a function of the distance (μm). Two curves (red and green) overlap, indicating that difference in their molecular masses and structure had little effect on the profile of the gradient under our experimental condition.

FigS3. The biphasic dynamics of PH_{Crac} -GFP translocation in a cell suddenly exposed to a static cAMP gradient (another example of experiments shown in Fig. 6).

(A) A PH cell (green) is suddenly exposed to a cAMP gradient at 8.0 s (red). Membrane translocation of PH_{Crac} -GFP occurs everywhere in the inner cell membrane (19.5s). Membrane-bound PH_{Crac} -GFP declined in both the front and back of the cell (71.1s), and a second increase only occurred in the front side (104.4s). Front (FD) and back (BD) regions used to evaluate changes of Alexa 594 intensity as a measure of cAMP concentration. FPH and BPH were selected membrane regions used for monitoring the response of PH_{Crac} -GFP translocation to the front and the back of the cell relative to the cAMP gradient. (B). Rapid generation of a stable cAMP gradient. (C) Dynamic changes in PH_{Crac} -GFP membrane translocation at the front (FPH) and the back side (BPH) of the cell.

FigS4. G-protein activation following sudden exposure to a steady cAMP gradient (another example of the experiments shown in Fig. 7).

(A) Dynamics of PH_{Crac} -GFP membrane association in the front of a nearby PH cell. (B) G-protein activation in the front (black) and back (gray) regions of a G cell, shown as the temporal changes in ratio of CFP/YFP.

Table 1. cAMP-dose-dependent T_{max} and R_{max} of transient PH_{Crac} -GFP responses from multiple cells in a uniform field of cAMP