digested by an 8-bit analog-to-digital converter (Ma- 
trox) installed on a 486-66 PC. Initially, a reference 
map of the blood vessel pattern at the surface of the 
choroid plexus was acquired using low light filtered 
at 550 ± 40 nm (Ealing). The camera was then focused 
300 μm below the surface of the cortex. Light from a 
100-W tungsten-halogen light source driven by a dc 
power supply (Kepco) was passed through a 610-
nm filter and used to illuminate the cortex during data 
collection. Frames were summed between 0.9 to 
3.6 s after stimulus onset, corresponding to the time 
of maximum signal as determined by our previous 
experiments (26). Data were analyzed with the use of 
in-house programs written in C++ (Bortland and IDL 
Research Systems).

8. Square-wave luminance gratings (typical parameters for 
V2: spatial frequency, 0.15 cycle per degree; drift velo-
city, 12° per second; 64% contrast) and subjective grat-
ings (typical spatial frequency of subjective orientation, 
0.15 cycle per degree; spatial frequency of inducing 
lines, 0.45 cycle per degree; drift velocity, 12° per sec-
ond for V2) were shown to the animal on a 17-inch 
monitor positioned 28.5 cm in front of it. In some ex-
periments, the spatial frequency of subjective edges was 
systematically varied. Neutral gray intensity was 6.0 cd/ 
m². All stimuli were shown binocularly. Subjective grat-
ing stimuli of four different orientations (0°, 45°, 90°, 
and 135°) were randomly interleaved with square-wave lu-
nance grating stimuli and presented 80 to 100 times. 
Luminance-defined inducing lines, 1 to 2 pixels wide, 
were used as the subjective orientations for sub-
jective grating stimuli. Gratings were drifted normal 
to the subjective edge orientation and parallel to the orien-
tation of the inducing lines in both directions separately. 
Eyes were closed. Central foveal stimuli were 
checked at the start of imaging by use of a reverse 
ophthalmoscope to project an image of the retinal vas-
culature onto the screen.

9. Orientation maps obtained with luminance gratings 
composed of thin lines (of the same width as the sub-
jective grating inducing lines) were identical to ori-
entation maps obtained with thicker bars (Fig. 2C) and 
regular grating fundamental spatial frequency. 

10. We compared orientation strengths (magnitudes of 
the orientation difference). The mean orientation 
strength for subjective gratings (1.00 ± 10-3 units) was 
slightly higher than the mean orientation strength for luminance gratings (0.87 ± 10-3 units) for these pixels. Thus, the response to subjective grating in V1 was stronger for subjective gratings than for luminance gratings for this subset of pixels, although they occupied a small portion of V1.

17. Receptive field properties of a subset of V1 cells 
whose luminance and subjective orientation prefer-
ences differed by ±45° or less were studied in detail. 
Three cells were complex; one was a simple cell. 
Two cells were end-stopped, four cells were non-
end-stopped. Six cells whose locations were identi-
fied were encountered at depths ranging from 300 to 
1200 μm from the surface. Among these responses 
were found those with the optical images region 
that responded preferentially to the slower drift rate, higher spatial frequency stimulus (V1) compared to the higher drift rate, lower spatial frequency stimu-
lus (V2), we could locate the V1/V2 boundary accu-
rately. The physiological border coincided with the 
anatomical border between areas 17 and 18 as de-
monstrated by marker lesions and histology.

18. Responses to subjective gratings cannot be explained 
by responses to line terminations. We have shown that 
both luminance and subjective gratings may respond 
optimally to a grating composed of a grid of dots (line ends) with the same parameters (spatial frequency, temporal fre-
quency) as the subjective grating but of an entirely dif-
ferent orientation. Some cells that are tuned to the 
same orientation of luminance and subjective gratings 
were found that respond stronger than the cell dot 
dot grating. A response to Fourier energy alone cannot explain 
why the response of a cell to an intermediate subjective orientation is higher than the response at 90° orientation (difference of the inducing orientations in the sub-
jective grating), because the energy along these orien-
tations is less than the energy along the inducing-line 
orientation. Moreover, most such cells respond optimal-
ly to a single intermediate orientation, despite the pres-
ence of equal Fourier energy in the stimulus along both 
the optimal orientation and its orthogonally oriented 
counterpart.

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well: V1 receives input from X and Y cells located in 
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Requirement of CDC42 for Salmonella-Induced 
Cytoskeletal and Nuclear Responses

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The bacterial pathogen *Salmonella typhimurium* triggers host cell signaling pathways that lead to cytoskeletal and nuclear responses required for pathogenesis. Here, the role of the small guanosine triphosphate (GTP)-binding protein CDC42Hs in these responses was examined. Expression of a dominant interfering mutant of CDC42 (CDC42HsN17) prevented *S. typhimurium*-induced cytoskeletal reorganization and subsequent macropinocytosis and bacterial internalization into host cells. Cells expressing constitutively active CDC42 (CDC42HsV12) internalized an *S. typhimurium* mutant unable to trigger host cell responses. Furthermore, expression of CDC42HsN17 prevented *S. typhimurium*-induced JNK kinase activation. These results indicate that CDC42 is required for bacterial invasion and induction of nuclear responses in host cells.

Interaction of the bacterial pathogen *Sal-
monella typhimurium* with host cells acti-
vates a bacterially encoded protein secre-
tion system that directs the export and, in 
some cases, the translocation into the host 
cell of several bacterial proteins (1). These 
proteins, in turn, trigger signal transduction 
pathways that lead to a variety of cellular 
responses. Among these responses is an extensive 
reorganization of the actin cytoskeleton, re-

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resulting in morphological changes that resemble the membrane ruffles induced by growth factors, hormones, and activated oncogenes (2). These cytoskeletal rearrangements are accompanied by macropinocytosis (3) and ultimately result in bacterial internalization into the cell. The ability of Salmonella to enter nonphagocytic cells is essential to pathogenesis as it allows the bacteria to breach the intestinal wall or to gain access to the intracellular environment where the host defense mechanisms can be more effectively evaded.

Previous studies have established that Salmonella can induce complex signaling pathways in cultured epithelial cells (4, 5). Although the understanding of these signaling events is incomplete, it is clear that the bacteria are similar to other well-characterized agonists in eliciting both cytoskeletal reorganization and induction of gene expression.

A group of low molecular weight GTP-binding proteins (Cdc42, Rac, Rho) have been shown to coordinate signaling cascades that produce both morphological and nuclear responses to a variety of extracellular signals. In these cascades, in which one guanosine triphosphatase regulates the action of the next, Cdc42 controls the formation of filopodia; Rac controls the subsequent formation of lamellipodia and membrane ruffling; and Rho controls the formation of stress fibers and focal adhesions (6). We examined whether these small GTP-binding proteins contribute to the Salmonella-induced cellular responses. Mutants of CDC42Hs were expressed in COS-1 cells using a selection system (7) in which the cells expressing the different CDC42Hs proteins could be identified by the coupled expression of the green fluorescent protein (GFP). We transfected COS-1 cells with plasmids encoding various CDC42Hs mutants, infected the cells with wild-type S. typhimurium, and then looked for macropinosomes resulting from bacterially induced membrane ruffling (8) (Fig. 1, A and B). Alternatively, we fixed and treated infected cells with rhodamine-labeled phalloidin, which stains polymerized actin and therefore can reveal cytoskeletal changes (9) (Fig. 2, A and B), or we quantified bacterial internalization (10).

Expression of a dominant-negative mutant of CDC42Hs (CDC42HsN17) abrogated the cytoskeletal rearrangements (Fig. 2, B and C) and the formation of macropinosomes (Fig. 1) induced by S. typhimurium infection and prevented bacterial internalization into trans-

![Fig. 1. Requirement of CDC42 for Salmonella-induced macropinocytosis. COS-1 cells were transfected with vectors coding for CDC42Hs mutants and GFP. (A) Transfected cells were infected with wild-type strain SL1344 for 1 hour and examined by phase and fluorescence microscopy. (B) Quantitation of transfected cells undergoing macropinocytosis as a consequence of infection. The n values are the number of cells examined and represent a combination of at least five experiments. (C) Expression levels of the different CDC42 mutants in the transfected cells, as determined by immunoblot analysis.](http://science.sciencemag.org/lookup/fig/1)

![Fig. 2. Requirement of CDC42 for Salmonella-induced cytoskeletal rearrangements and bacterial internalization. COS-1 cells were transfected with vectors coding for CDC42N17 and GFP, infected with SL1344, and stained with rhodamine-labeled phalloidin to visualize F-actin (A to C) or examined for the presence of internalized bacteria (D). (A) Vector-transfected cells before (1) and after (2 and 3) infection. Panels 1 and 2 show composites of images obtained with fluorescein isothiocyanate (FITC) and rhodamine filters. Panel 3 shows the same image as panel 2 but obtained with the FITC filter alone. (B) Cells expressing CDC42N17 and GFP before (1) and after (2) infection. Photomicrographs are composites of images obtained with FITC and rhodamine filters. (C) Quantitation of transfected cells with cytoskeletal rearrangements resulting from infection. (D) Quantitation of bacterial invasion (10). The n values are the number of cells examined for each category and represent a combination of at least five experiments.](http://science.sciencemag.org/lookup/fig/2)
fected cells (Fig. 2D). A small number of cells expressing CDC42HsN17 responded to infection but these cells had few macropinosomes, minor cytoskeletal rearrangements, and few internalized bacteria (less than three bacteria per cell). In contrast, expression of wild-type CDC42Hs, a prenylation-defective form (CDC42HsS188), a constitutively active form (CDC42HsL61), or GFP alone had no effect on the infection-induced cytoskeletal rearrangements and macropinocytosis (Figs. 1 and 2, A to C) and bacterial internalization (Fig. 2D). Similar results were obtained with infected HeLa cells (11).

To test whether expression of a constitutively active form of CDC42Hs confers on the cell the ability to internalize an invasion-defective mutant of S. typhimurium, we infected Rat-1 cells stably expressing CDC42HsV12 (12) with the noninvasive strain SB136. This strain carries a nonpolar mutation in the invA gene, which encodes an essential component of the invasion-associated type III protein secretion system, and therefore cannot initiate host cell signaling (13). SB136 was internalized by Rat-1 CDC42HsV12 stable transfectants but not by the control Rat-1 cells stably transfected with the vector alone (Fig. 3), indicating that activation of CDC42Hs can rescue the invasion phenotype of the invA mutant. Similar results were obtained with COS-1 cells transiently expressing the constitutively active mutant CDC42L61 (11).

We next investigated whether Rac1 plays a role in the Salmonella-induced cytoskeletal rearrangements and bacterial internalization. COS-1 cells were transfected with an expression vector coding for Rac1 mutants (7), infected with wild-type S. typhimurium, and examined for macropinosomes (8), cytoskeletal changes (9), and bacterial internalization (10). Expression of a dominant interfering mutant of Rac1 (Rac1N17) modestly inhibited the bacterially induced changes, but to a much lesser extent than did CDC42HsN17 (Fig. 4). In contrast, expression of wild-type Rac1, a constitutively active form (Rac1V12), or the vector alone had no effect on bacterial internalization, cytoskeletal rearrangements, or macropinocytosis. A previous study indicated that Rac1 is not required for Salmonella-induced cytoskeletal rearrangements in Swiss 3T3 cells (14). This discrepancy may be due to the fact that we examined a larger number of cells, perhaps allowing detection of more subtle effects.

We have shown that S. typhimurium activates several transcription factors that are involved in the production of pro-inflammatory cytokines such as interleukin 8, and that these nuclear responses require activation of the MAP kinases JNK and p38 (15). It has been reported that constitutively active CDC42 leads to the activation of the JNK and p38 MAP kinases (16). We therefore tested the possibility that CDC42 is required for Salmonella-induced activation of JNK (17). Expression of a dominant interfering mutant of CDC42 (CDC42HsN17) prevented bacterially induced JNK activation (Fig. 5), suggesting that CDC42 is required for these nuclear responses.

The mechanisms by which CDC42 is engaged in the cellular responses to infection are not known. The strict dependence of the responses on the bacterial Type III protein secretion system suggests that translocation of a bacterial effector protein (proteins) into the host cell may stimulate the signaling pathways leading to CDC42 activation. Previous work implicating arachidonic acid metabolites in Salmonella-induced cytoskeletal changes (5) and Rho family function (18) raises the possibility that this cellular signaling pathway may be important.

Our results indicate that CDC42 induces cytoskeletal rearrangements independently of Rac. Although membrane ruffling has been associated more frequently with Rac activity, the relative importance of the different G proteins in modulating cytoskeletal rearrangements may largely depend on the agonist and cell type (19). Potential
targets of CDC42 that may largely play a role in these responses include a group of protein kinases with homology to the yeast Ste20 protein (PAK kinases), the Wiskott-Aldrich syndrome protein (WASP), and phosphatidylinositol-3-kinase (PI3-kinase) (20). PI3-kinase is unlikely to play a role in S. typhimurium–induced signaling, however, as wortmannin, a potent inhibitor of PI3-kinase, has no effect on the S. typhimurium–induced cell responses (11).

REFERENCES AND NOTES

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8. COS-1 cells grown in 6-cm culture dishes were transfected with 1 μg of pcDNA-FLAG-Jnk-1, which encodes a FLAG epitope–tagged Jnk-1, and 2 μg of either pcDNA3CD42HsN17 or the vector pcDNA3. After 48 hr, cells were mock-infected or infected with strain SL1344 for 30 min at an MOI of 20 in DMEM. The Jnk kinase activity in cell lysates was determined as described previously [S. Bagrodia, B. Derijard, R. J. Davis, R. A. Cerione, J. Biol. Chem. 270, 27995 (1995)].
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TECHNICAL COMMENTS

Consequences of Retinal Color Coding for Cortical Color Decoding

Dennis M. Dacey et al. in their report (1) and Richard H. Masland in his Perspective (2) draw attention to important details in the encoding of color in the retina of macaque monkeys and humans. The centers of red-green opponent retinal ganglion cells can be driven by a single cone, but the cone specificity of the surrounds is in question. Dacey et al. state that horizontal cells that subserve red-green opponent ganglion cells are contacted by both L- and M-cones, a finding with implications for receptive field formation (1), retinal coding (1, 2), and cortical decoding (2). While Dacey et al. may well be correct that surrounds are coded by post-horizontal cell processes, I question whether mixed cone surrounds pose insurmountable problems for retinal color coding or cortical decoding. The color signals of units with mixed cone surrounds are less complicated if the spatial properties of the units are taken into account using the Ingling-Martinez identity (3)—a rigorous statement of the co-coding hypothesis discussed by Masland. Let x be the weight of a P cell–L-cone center and y and z be the weights of M- and L-cones driving the surround. The Ingling-Martinez identity that describes this P cell is

\[ xL + yM + zS = 0.5(x + y + yM)(C - S) + 0.5(y - yL - yM)(C + S) \]

where C and S are center and surround spatial weighting or modulation transfer functions. In this equation, the first term represents the bandpass spatial response to achromatic stimuli and the second term, the lowpass spatial response to chromatic stimuli. If \( z = 0 \), then the surround is pure, and the cone weighting of the achromatic and chromatic responses differ only in polarity. The effect of mixed cone surrounds is to give the achromatic and chromatic responses different cone weightings (4). This is the case psychophysically—for the CIE standard observer, the achromatic response is approximately 5L3M, while the red-green color response is 2L3M. Reconciling these different weights using pure surrounds has motivated several models (5). Mixed surrounds can yield this result directly [that is, if \( (x, y, z) = (3.5, 3.0, 1.5) \) and is roughly what would be expected (6) for random surrounds constructed on an L-cone rich–retina (such as that posited to underlie the standard observer’s luminosity function).

Do mixed cone surrounds pose difficulties for cortical color/luminance decoding? Recent models of achromatic/chromatic demultiplexing rely on spatial filtering operations that are based on the spatial properties of the center/surround combinations in Eq. 1, but are robust with respect to surround cone ratios (4, 7–9). Filtering models have no problem accounting for the major red-green cell classes in cytochrome oxidase blobs; type II cells, \( n \) double-opponent cells, and double-opponent cells can be created from filtering operations on parvo cells (8). Similar models account for extraction of achromatic information (4, 7, 9). These filtering operations do not always create a
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