measures are an important component of control strategies.

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Neuronal GPCR Controls Innate Immunity by Regulating Noncanonical Unfolded Protein Response Genes

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The unfolded protein response (UPR), which is activated when unfolded or misfolded proteins accumulate in the endoplasmic reticulum, has been implicated in the normal physiology of immune defense and in several human diseases, including diabetes, cancer, neurodegenerative disease, and inflammatory disease. In this study, we found that the nervous system controlled the activity of a noncanonical UPR pathway required for innate immunity in *Caenorhabditis elegans*. OCTR-1, a putative octopamine G protein–coupled catecholamine receptor (GPCR, G protein–coupled receptor), functioned in sensory neurons designated ASH and ASI to actively suppress innate immune responses by down-regulating the expression of noncanonical UPR genes *pqn/abu* in nonneuronal tissues. Our findings suggest a molecular mechanism by which the nervous system may sense inflammatory responses and respond by controlling stress-response pathways at the organismal level.

Endoplasmic reticulum (ER) stress has been linked to several human diseases, including diabetes, cancer, neurodegenerative disease, and inflammatory disease (*1*–*3*). The ER has developed specific signaling pathways, known as the unfolded protein response (UPR), to cope with ER stress and restore ER homeostasis. Recent studies indicate that increased demand on protein folding in the ER, which may occur during bacterial infections, must be alleviated by UPR pathways for a complete immune response to be mounted (*4*–*8*).

We took advantage of the simple nervous and immune systems of the nematode *Caenorhabditis elegans* to investigate the role of the nervous system in the organismal control of pathways involved in innate immune responses. Three sensory neurons (AQR, PQR, and URX) are known to regulate resistance to pathogen infections by controlling the activation of a p38 mitogen-activated protein kinase (MAPK) pathway and the *C. elegans* avoidance to certain pathogens (*9*, *10*). In addition, a range of chemosensory neurons that penetrate the cuticle and directly detect and respond to different environmental cues have the potential to control innate immune responses.

To elucidate the molecular mechanism by which these neurons regulate innate immunity in response to pathogen infection, we first determined the susceptibility to the human opportunistic pathogen *Pseudomonas aeruginosa* of *octr-1(ok371)* animals, which lack a G protein–coupled catecholamine receptor normally expressed in the cilia of neurons located in sensory openings, including ASH, ASI, AIY, and dopaminergic ADE/CEP neurons (*11*, *12*). *octr-1(ok371)* animals exhibited enhanced resistance to killing by *P. aeruginosa* (Fig. 1A), which suggests that the loss of OCTR-1 signaling increases the general immune function of the nematodes. We observed no difference in survival between *octr-1(ok371)* and wild-type (WT) animals that were fed heat-killed *P. aeruginosa* or *Escherichia coli* (Fig. 1B and fig. S1). Thus, *octr-1* mutation affects the immune response to living pathogenic bacteria without altering the basic life span of the nematodes.

Because pathogen avoidance is part of the *C. elegans* defense response to *P. aeruginosa* (*9*), we examined the susceptibility to *P. aeruginosa*–mediated killing of *octr-1(ok371)* animals on agar plates that were completely covered in bacteria, a condition that eliminates pathogen avoidance. *octr-1(ok371)* animals died at a slower rate than did WT animals (Fig. 1C), indicating that pathogen avoidance does not play a role in *octr-1(ok371)*–enhanced resistance to *P. aeruginosa.* In addition, the magnitude of pathogen avoidance of *octr-1(ok371)* and WT animals was similar (Fig. 1D). The enhanced resistance of *octr-1(ok371)* to *S. enterica* (fig. S2), a pathogen that does not elicit an avoidance behavior (*13*), further supports the function of OCTR-1 in the regulation of immune responses.

Certain mutants resistant to pathogen infection exhibit resistance to pathogen accumulation (*14*). Therefore, we examined whether *octr-1* mutation affected bacterial accumulation in the intestine. Compared to WT animals, *octr-1(ok371)* animals exhibited a similar accumulation pattern of *P. aeruginosa*/green fluorescent protein (GFP) or *S. enterica*/GFP (Fig. 1E and fig. S3). In addition, the number of bacterial cells in *octr-1(ok371)* animals was similar to that in WT animals (Fig. 1F and fig. S4). Thus, the bacterial load to which the animals were exposed was comparable, and reduced bacterial accumulation levels in the intestine did not contribute to the enhanced immunity of *octr-1(ok371)* animals, indicating that *octr-1(ok371)* animals exhibit enhanced endurance to *P. aeruginosa* infection.

To provide insights into the mechanism underlying the enhanced immunity of *octr-1(ok371)* ani-

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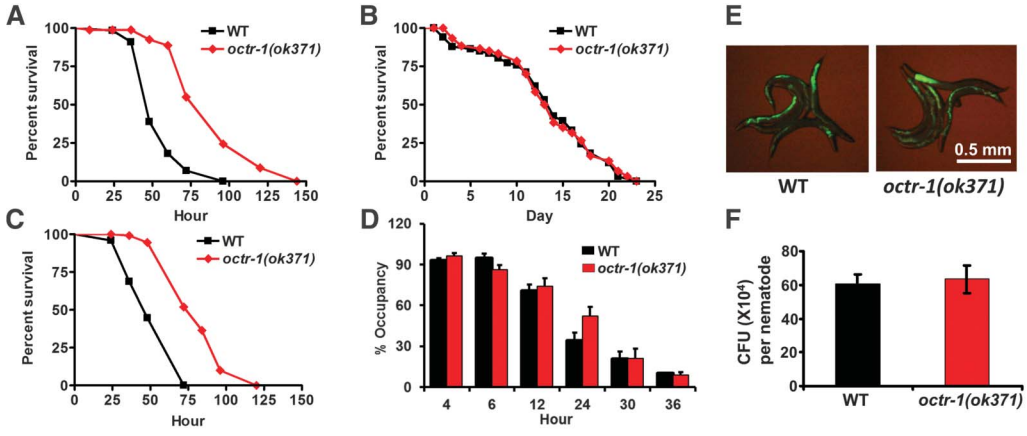

Fig. 1. *C. elegans* G protein–coupled receptor OCTR-1 is involved in immunity to *P. aeruginosa*. (**A**) WT and *octr-1(ok371)* animals were exposed to *P. aeruginosa*. (**B**) WT and *octr-1(ok371)* animals were exposed to heat-killed *P. aeruginosa*. (**C**) WT and *octr-1(ok371)* animals were exposed to a full lawn of *P. aeruginosa*. The survival graphs represent combined results of two independent experiments. $n = 90$ adult animals per strain. (**D**) Animals were placed on a small spot of *P. aeruginosa* in a 3.5-cm plate and monitored over time for their presence or absence on the lawn. The graph represents combined results of three independent experiments. $n = 60$ adult animals per strain. (**E**) WT and *octr-1(ok371)* animals were exposed to *P. aeruginosa* expressing GFP for 48 hours and then visualized using a MZ FLIII Leica stereomicroscope. (**F**) WT and *octr-1(ok371)* animals were exposed to *P. aeruginosa* expressing GFP for 48 hours, and the colony forming units (CFU) were quantified. Ten animals were used for each condition. The graph represents combined results of three independent experiments. Bars represent mean ± SEM.

mals, we used genome microarrays to find clusters of genes commonly misregulated in *octr-1(ok371)* relative to WT animals grown on live *P. aeruginosa*. OCTR-1–regulated genes previously known to be regulated by pathways involved in *C. elegans* innate immunity—including a DAF-2/DAF-16 insulin pathway, a p38/PMK-1 MAPK pathway, and a CED-1 pathway—were enriched (Fig. 2A and tables S1 and S2). Unexpectedly, the enhanced resistance to *P. aeruginosa*–mediated killing of *octr-1(ok371)* animals was suppressed by *pmk-1* RNA interference (RNAi) or mutation, but not by *daf-16* RNAi or mutation (Fig. 2, B and C, and fig. S5). Mutation in *sek-1*, which encodes for a MAPK kinase essential for PMK-1 activation, also suppressed the enhanced resistance of *octr-1(ok371)* animals (fig. S6). Consistent with the idea that derepression of PMK-1 transcriptional activity by lack of OCTR-1 signaling activates innate immunity, *octr-1(ok371)* animals exhibited higher levels of active PMK-1 than WT animals (Fig. 2D). Thus, in contrast to AQR, PQR, and URX neurons, which control not only PMK-1 activity but also pathogen avoidance, OCTR-1–expressing neurons control PMK-1 activity without affecting pathogen avoidance.

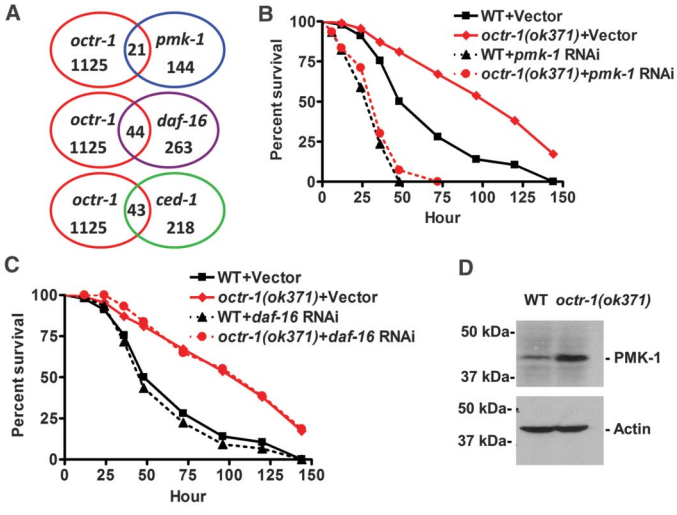

Fig. 2. OCTR-1 controls pathways required for *C. elegans* innate immunity. (**A**) Venn diagrams of genes that are up-regulated in *octr-1(ok371)* animals and positively regulated by *daf-16*, *pmk-1*, and *ced-1*. (**B**) WT and *octr-1(ok371)* animals grown on double-stranded RNA (dsRNA) for vector control or dsRNA for *pmk-1* were exposed to *P. aeruginosa*. (**C**) WT and *octr-1(ok371)* animals grown on dsRNA for vector control or dsRNA for *daf-16* were exposed to *P. aeruginosa*. The survival graphs represent combined results of two independent experiments. $n = 90$ adult animals per strain. (**D**) Active PMK-1 was detected in WT and *octr-1(ok371)* animals. Animals were grown at 20°C until 1-day-old adult and whole-worm lysates were used to detect active PMK-1 with an antibody against human p38 from Promega (Madison, WI). Actin was used as loading control. kDa, kilodaltons.

Among genes up-regulated in *octr-1(ok371)* animals, a group of 43 genes transcriptionally regulated by the phagocyte receptor CED-1 (*4*) was significantly enriched (Fig. 2A and table S2). Indeed, 7 out of 14 genes (Fig. 3A) that were up-regulated greater than twofold in *octr-1(ok371)* animals correspond to a family of *pqn* (*prionlike glutamine[Q]/asparagine[N]-rich domain-bearing protein*) genes that are controlled by CED-1 (*4*). Eleven genes in the *pqn* family are classified as *abu* (*activated in blocked unfolded protein response*) (*15*), because they are activated in *xbp-1* mutant animals when ER stress is induced by tunicamycin treatment. The *abu* genes encode UPR proteins that function in parallel with the canonical UPR pathway (*15*). We studied whether the most highly up-regulated *pqn* genes in *octr-1(ok371)* mutants could be induced by tunicamycin in an *xbp-1* mutant background. Indeed, *pqn-40*, *pqn-46*, *pqn-95*, and *pqn-5* genes were found to be activated by ER stress in animals with blocked UPR (table S3) and were named *abu-12*, *abu-13*, *abu-14*, and *abu-15*. *abu-1*, *-7*, *-8*, *-12*, *-13*, *-14*, and *-15* were significantly up-regulated in *octr-1(ok371)* animals (Fig. 3B), suggesting that the noncanonical UPR pathway is inhibited by OCTR-1. Consistent with this idea that *abu* genes encode noncanonical UPR proteins that may interact with abnormal ER client proteins, ABU-1 is retained in the ER by its transmembrane domain (*15*). Furthermore, with the exception of *pqn-46*, all *pqn/abu* genes that were up-regulated in *octr-1(ok371)* animals encode proteins that have both an ER signal sequence and a transmembrane domain (table S4).

Nine out of thirteen *pqn/abu* genes up-regulated in *octr-1(ok371)* animals are strongly expressed in the pharynx and the intestine, primary interfaces between host cells involved in immune responses and bacterial pathogens. Transgenic animals show expression of ABU-1::GFP in vesicular structures corresponding to the pharyngeal cells (*15*). In situ hybridizations completed as part of NEXTDB (The Nematode Expression Pattern Database) reveal expression of eight *pqn/abu* genes in the pharynx and the intestine from L3 to adult stages (http://nematode.lab.nig.ac.jp). To investigate the relation between the noncanonical UPR pathway and host responses to *P. aeruginosa* infection, we tested whether exposure to this pathogen would up-regulate *abu* genes. Indeed, *P. aeruginosa* exposure up-regulated *abu* genes (fig. S7 and table S5), indicating that the noncanonical UPR pathway may be required for *C. elegans* immune response against this pathogen. We then studied the resistance to *P. aeruginosa*–mediated

killing of WT and *octr-1(ok371)* animals where *abu* genes were inhibited by RNAi. Inhibition of *abu-1*, *-7*, *-8*, *-12*, and *-13* partially suppressed the enhanced immunity of *octr-1(ok371)* animals (Fig. 3C). RNAi-mediated inhibition of *abu* genes also resulted in enhanced susceptibility to *P. aeruginosa*–mediated killing (fig. S8).

Because *ced-1* is part of a pathway that transcriptionally controls the expression of *pqn/abu* genes (*4*), we asked whether *ced-1* mutation suppresses the enhanced immunity of *octr-1(ok371)* animals. The survival of *octr-1(ok371);ced-1(e1735)* animals was comparable to that of WT animals (Fig. 3D). We observed no additive effect of *abu* RNAi ablation on *octr-1(ok371);ced-1(e1735)* animals (Fig. 3E), confirming that *ced-1* and *abu* genes are part of the same pathway responsible for the enhanced resistance to pathogen infection of *octr-1(ok371)* animals. Furthermore, *abu-1* overexpression protected *octr-1(ok371);ced-1(e1735)* animals from *P. aeruginosa*–mediated killing (Fig. 3F). Thus, higher CED-1–mediated signaling is responsible for the enhanced resistance to *P. aeruginosa*–mediated killing in animals with impaired neural function due to mutation in *octr-1*.

To determine the specific foci of OCTR-1 activity involved in the control of innate immunity, we studied the pathogen susceptibility of *octr-1(ok371)* animals expressing *octr-1* under the control of the *sra-6* promoter, which drives the expression of *octr-1* to the ASH, ASI, and PVQ neurons (*16*). Expression of *octr-1* under the control of the *sra-6* promoter fully rescued the mutant phenotype of *octr-1(ok371)* animals (Fig. 4A), indicating that OCTR-1–expressing neurons regulate innate immunity in *C. elegans*. Consistent with this idea, OCTR-1 expression under the regulation of its own promoter also fully rescued the mutant phenotype of *octr-1(ok371)* animals (Fig. 4B). Because PVQ neurons do not express *octr-1*, our results suggest that ASH and ASI neurons negatively regulate innate immunity. Thus, we studied the susceptibility to *P. aeruginosa* of a strain in which mutation in *polyQ enhancer-1* promotes early onset degeneration of ASH neurons caused by polyQ toxicity (*17*). The strain lacking ASH neurons exhibited a significantly increased survival on *P. aeruginosa* (fig. S9), indicating that ASH neurons suppress innate immunity.

We confirmed that ASH and ASI neurons negatively regulate *abu* genes required for appropriate innate immunity by studying the gene expression levels of *abu-1*, *-12*, *-13*, *-14*, and *-15* in WT, *octr-1(ok371)*, and *octr-1(ok371)* animals expressing *octr-1* under the control of the *sra-6* promoter. Whereas the expression levels of *abu* genes in *octr-1(ok371)* animals are higher than those in WT animals, their expression levels are close to those of the wild type in *octr-1(ok371)* animals where *octr-1* expression in ASH and ASI was restored (Fig. 4C).

We have shown that OCTR-1–expressing neurons ASH and ASI negatively regulate innate immunity, at least in part, by controlling the expression of a family of genes that are part of a noncanonical UPR pathway regulated by the

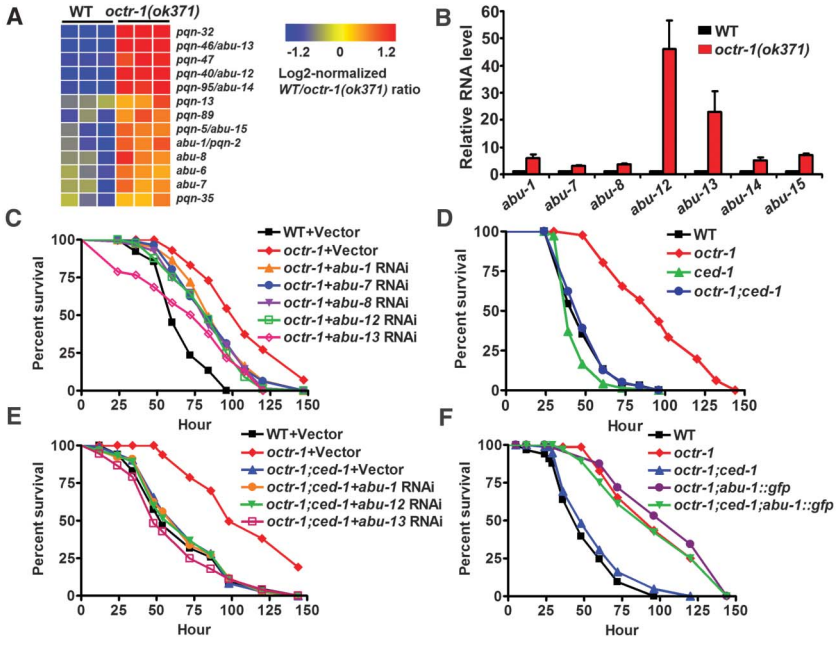

Fig. 3. *pqn/abu* genes are required for the enhanced immunity of *octr-1(ok371)* animals to *P. aeruginosa*. (**A**) Cluster of *pqn/abu* genes that are up-regulated in *octr-1(ok371)* mutants. (**B**) Quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis of *abu-1*, *abu-7*, *abu-8*, *abu-12*, *abu-13*, *abu-14*, and *abu-15* expression in *octr-1(ok371)* relative to WT animals exposed to *P. aeruginosa*. $n = 3$ independent experiments; bar graphs correspond to mean ± SEM (error bars). (**C**) WT animals grown on dsRNA for vector control and *octr-1(ok371)* animals grown on dsRNA for vector control or dsRNA for *abu* genes were exposed to *P. aeruginosa*. (**D**) WT, *octr-1(ok371)*, *ced-1(e1735)*, and *octr-1(ok371);ced-1(e1735)* animals were exposed to *P. aeruginosa*. (**E**) WT and *octr-1(ok371)* animals grown on dsRNA for vector control and *octr-1(ok371);ced-1(e1735)* animals grown on dsRNA for vector control or dsRNA for *abu* genes were exposed to *P. aeruginosa*. (**F**) WT, *octr-1(ok371)*, *octr-1(ok371);ced-1(e1735)*, *octr-1(ok371);abu-1::gfp(zcEx8)*, and *octr-1(ok371);ced-1(e1735);abu-1::gfp(zcEx8)* animals were exposed to *P. aeruginosa*. The survival graphs represent combined results of two independent experiments. $n = 90$ adult animals per strain.

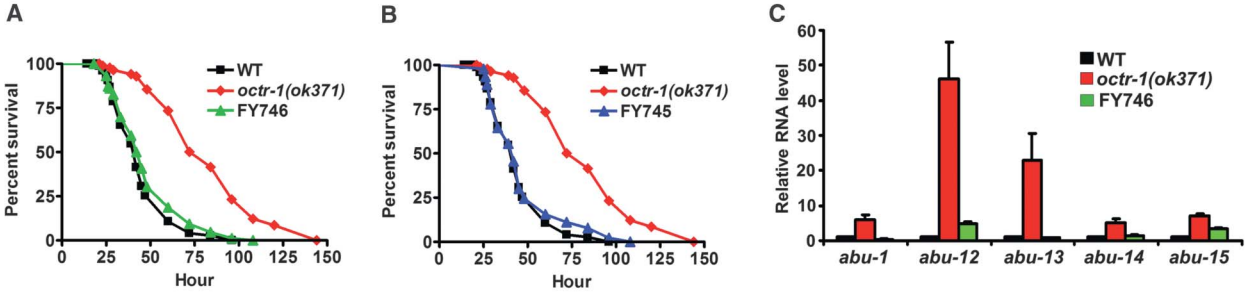

Fig. 4. OCTR-1–expressing neurons ASH and ASI suppress innate immunity by controlling the expression of *pqn/abu* genes. (**A**) WT, *octr-1(ok371)*, and FY746 *octr-1(ok371);grEx158[Psra-6::octr-1]* animals were exposed to *P. aeruginosa*. (**B**) WT, *octr-1(ok371)*, and FY745 *octr-1(ok371);grEx157 [Poctr-1::octr-1::gfp]* animals were exposed to *P. aeruginosa*. The survival graphs represent the combined results of two independent experiments. $n = 90$ adult animals per strain. (**C**) Quantitative RT-PCR analysis of *abu-1*, *abu-12*, *abu-13*, *abu-14*, and *abu-15* expression in *octr-1(ok371)* and FY746 *octr-1(ok371);grEx158[Psra-6::octr-1]* relative to WT animals exposed to *P. aeruginosa*. $n = 3$ independent experiments; bars represent mean ± SEM.

apoptotic receptor CED-1. Furthermore, the conserved p38 MAPK pathway is under the control of OCTR-1–expressing neurons. Recent studies indicate that the murine nervous system, through the vomeronasal organ, has the potential to "smell" molecules related to disease or inflammation in the outside world (*18*). Even though the mammalian nervous system plays a key role in the regulation of inflammation (*19*), the specific role of neurons in contact with the outside world in the control of immune responses remains unclear.

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Supporting Online Material

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Transient Activation of the HOG MAPK Pathway Regulates Bimodal Gene Expression

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Mitogen-activated protein kinase (MAPK) cascades are conserved signaling modules that control many cellular processes by integrating intra- and extracellular cues. The p38/Hog1 MAPK is transiently activated in response to osmotic stress, leading to rapid translocation into the nucleus and induction of a specific transcriptional program. When investigating the dynamic interplay between Hog1 activation and Hog1-driven gene expression, we found that Hog1 activation increases linearly with stimulus, whereas the transcriptional output is bimodal. Modeling predictions, corroborated by single-cell experiments, established that a slow stochastic transition from a repressed to an activated transcriptional state in conjunction with transient Hog1 activation generates this behavior. Together, these findings provide a molecular mechanism by which a cell can impose a transcriptional threshold in response to a linear signaling behavior.

Mitogen-activated protein kinase (MAPK) cascades orchestrate many cellular processes including cell growth, division, and differentiation (*1*). In *Saccharomyces cerevisiae*, the high osmolarity glycerol (HOG) pathway is needed to reestablish the balance between internal and external pressures upon osmotic shock (*2*). Osmosensors at the cell membrane activate either the MAPK kinase kinases (MAPKKKs) Ste11 or Ssk2,22, which converge on the MAPKK Pbs2. In turn, Pbs2 doubly phosphorylates the MAPK Hog1, leading to rapid translocation into the nucleus to launch a transcriptional program. Although increased transcription is essential to survive very high osmotic stress (0.8 M NaCl), it is not required for milder stress conditions (0.4 M NaCl) (*3*), under which Hog1 kinase activity alone is sufficient to drive cellular adaptation. By contrast, in the yeast mating MAPK pathway, transcription and new protein expression are required for cell cycle arrest and mating (*4*).

Transcriptional activation of mating genes occurs with linear kinetics and high fidelity (*5*, *6*), and the observed cell-to-cell variation in protein expression is governed by the ability of cells to express proteins (expression capacity) (*5*). Whereas the mating pathway can be compared to a cell-fate decision system with sustained MAPK activity, the HOG pathway is an adaptation response, which is only transiently induced like other stress-activated pathways (*7*). We therefore investigated whether this transient response would trigger different expression behavior.

To quantify the transcriptional output induced by osmotic stress, we engineered a reporter system based on a quadruple Venus (qV) fluorescent protein expressed under the control of specific osmostress-inducible promoters dependent on the three main transcription factors orchestrating the transcriptional response to osmotic stress (Hot1 and Sko1: p*STL1*; Msn2,4: p*ALD3*; or Msn2,4 and Hot1: p*HSP12*) (*8*). Flow cytometry revealed a Pbs2-dependent 20-fold increase in p*STL1*-qV reporter expression when 0.4 M NaCl was added to the growth medium (Fig. 1, A and B). No expression was detected at low salt concentrations (below 0.05 M), while above 0.15 M, all cells expressed the reporter and the amount of fluorescence increased linearly with stress. However, at intermediate concentrations, we observed histograms with two distinct subpopulations representing nonexpressing cells with basal autofluorescence levels and expressing cells with higher intensities. These distributions are termed bimodal. The p*ALD3*-qV and p*HSP12*-qV reporters displayed a similar bimodal expression behavior (Fig. 1B and fig. S1A). Induction of the mating pathway for 45 min with α-factor also generated a bimodal expression output of the Ste12-specific reporter p*FIG1*-qV. However, signaling in the mating pathway is prevented from "start" through S phase (*9*), and expression output became unimodal after relieving this cell cycle–dependent restriction (Fig. 1B and fig. S1B).

To investigate the source of the HOG pathway bimodal expression behavior, we integrated two reporters driving the expression of a quadruple cyan fluorescent protein (qCFP) and a qV construct in the same cell. Correlation of the cyan and yellow intensities measures the contribution of cell-to-cell (extrinsic) and intracellular (intrinsic) variability to the overall expression noise (*5*, *10*). The two p*FIG1* reporters induced by α-factor demonstrated that the mating pathway is governed by extrinsic noise. By contrast, we observed a lack of correlation between the two p*STL1* reporters (Fig. 1, C to E, and fig. S2), demonstrating that the bimodal expression behavior of the HOG pathway is independent of cell-to-cell variability caused by extrinsic factors such as expression capacity or cell-cycle stage.

To assess the observed bimodality and Hog1 signaling simultaneously, we combined a Hog1-relocation assay (*11*, *12*) with the p*STL1*-qV ex-

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