

**Science**

 AAAS

## Political Attitudes Vary with Physiological Traits

Douglas R. Oxley, *et al.*

*Science* **321**, 1667 (2008);

DOI: 10.1126/science.1157627

**The following resources related to this article are available online at [www.sciencemag.org](http://www.sciencemag.org) (this information is current as of October 31, 2008 ):**

**Updated information and services**, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/cgi/content/full/321/5896/1667>

**Supporting Online Material** can be found at:

<http://www.sciencemag.org/cgi/content/full/321/5896/1667/DC1>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

This article **cites 26 articles**, 3 of which can be accessed for free:

<http://www.sciencemag.org/cgi/content/full/321/5896/1667#otherarticles>

This article appears in the following **subject collections**:

Sociology

<http://www.sciencemag.org/cgi/collection/sociology>

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:

<http://www.sciencemag.org/about/permissions.dtl>

27. A. Iida, T. Nakamoto, H. Susa, *Icarus* **153**, 430 (2001).  
 28. F. H. Shu, H. Shang, A. E. Glassgold, T. Lee, *Science* **277**, 1475 (1997).  
 29. F. J. Ciesla, *Science* **318**, 613 (2007).  
 30. D. E. Brownlee *et al.*, *39th Lunar Planet. Sci. Conf. abstract 1978*; www.lpi.usra.edu/meetings/lpsc2008/pdf/1978.pdf (2008).  
 31. K. D. McKeegan *et al.*, *39th Lunar Planet. Sci. Conf. abstract 2020*; www.lpi.usra.edu/meetings/lpsc2008/pdf/2020.pdf (2008).
32. We thank K. Nakamura-Messenger, T. Iwazumi, Y. Wakabayashi, A. Koyama, T. Mori, Y. Suzuki, A. Takeuchi, Y. Terada, H. Nagahara, and H. Yoshida for technical support; M. Sekiya, H. Miura, and M. Uesugi for discussion; and KEK and SPring-8 for experiments. This work was supported by the Japan Society for the Promotion of Science, the NASA Stardust Sample Analysis, and Cosmochemistry Programs. The Wisconsin Secondary Ion Mass Spectrometer Laboratory is partly supported by NSF.

## Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5896/1664/DC1  
 Materials and Methods  
 SOM Text  
 Figs. S1 to S6  
 Tables S1 and S2  
 References

27 May 2008; accepted 19 August 2008  
 10.1126/science.1160995

## Political Attitudes Vary with Physiological Traits

Douglas R. Oxley,<sup>1\*</sup> Kevin B. Smith,<sup>1\*</sup> John R. Alford,<sup>2</sup> Matthew V. Hibbing,<sup>3</sup> Jennifer L. Miller,<sup>1</sup> Mario Scalora,<sup>4</sup> Peter K. Hatemi,<sup>5</sup> John R. Hibbing<sup>1†</sup>

Although political views have been thought to arise largely from individuals' experiences, recent research suggests that they may have a biological basis. We present evidence that variations in political attitudes correlate with physiological traits. In a group of 46 adult participants with strong political beliefs, individuals with measurably lower physical sensitivities to sudden noises and threatening visual images were more likely to support foreign aid, liberal immigration policies, pacifism, and gun control, whereas individuals displaying measurably higher physiological reactions to those same stimuli were more likely to favor defense spending, capital punishment, patriotism, and the Iraq War. Thus, the degree to which individuals are physiologically responsive to threat appears to indicate the degree to which they advocate policies that protect the existing social structure from both external (outgroup) and internal (norm-violator) threats.

The nature and source of political attitudes have been the subject of much study (1–3). Traditionally, such attitudes were believed to be built from sensible, unencumbered reactions to environmental events (4), but more recent research emphasizes the built-in, almost “automated” quality of many political responses (5), which has been suggested to be based in brain activation variations in limbic regions (6–8). The research task is now to determine why some people seem primed to adopt certain political attitudes, whereas others appear primed to adopt quite different attitudes. For example, although images and reminders of the terrorist attacks of 9-11 produce an aggregate shift in political views (9, 10), the reasons for individual variability in the degree of attitudinal shifts are unknown.

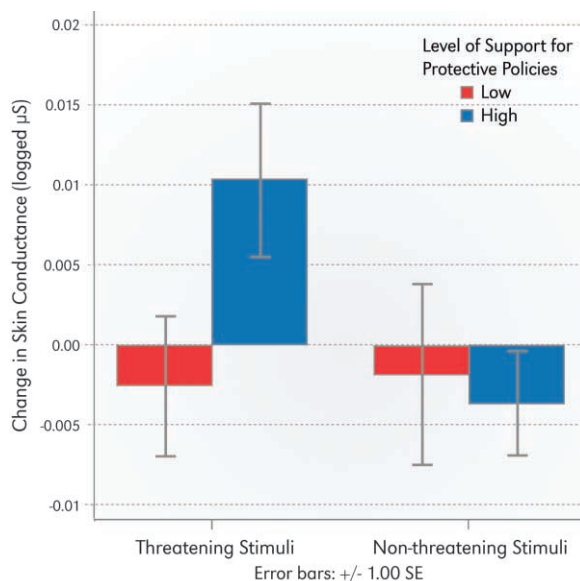
One possibility is that people vary in general physiology and that certain of these variations encourage the adoption of particular political attitudes. Broad, physiologically relevant traits such as feelings of disgust and fear of disease have been suggested to be related to political attitudes (11, 12), and political beliefs can be predicted by observing brain activation patterns in

response to unanticipated events, such as one letter of the alphabet appearing on a computer screen when the respondent expected a different letter (13). A connection between self-reports of felt threat and political attitudes has also been identified in previous research (14–19).

The physiology of response to a perceived threat is an attractive topic of investigation because an appropriate response to environmental threat is necessary for long-term survival and because perceived threat produces a variety of reasonably well-mapped, physically instantiated responses (20). If the threat is abrupt, a defensive cascade of linked, rapid extensor-flexor movement occurs

throughout the body within 30 to 50 ms (21), presumably to reduce vital-organ vulnerability (e.g., eye blink and retraction of the head). Less immediately, perceived threat causes signals from the sensory cortex to be relayed to the thalamus and ultimately to the brain stem, resulting in heightened noradrenergic activity in the locus ceruleus (22). Acetylcholine, acting primarily through the amygdala but also through the hypothalamic-pituitary-adrenal axis (23), stimulates release of epinephrine, which in turn leads to activation of the sympathetic division of the autonomic nervous system. Though these basic response patterns apply in all people, individual sensitivity to perceived threat varies widely (24).

To test the hypothesis that variations in physical sensitivity to threat are associated with political beliefs, in May 2007, we conducted a random telephone sample of the population of Lincoln, Nebraska. Participants were screened [see supporting online material (SOM)] to identify those with strong political attitudes (regardless of the content of those attitudes), and qualifying individuals were invited to a lab in the city. During the first visit, the 46 participants completed a survey instrument (see SOM) ascertaining their political beliefs, personality traits, and demographic characteristics. During the second session, about 2 months after the first, participants were attached to physiological equipment, making it possible to measure skin conductance and orbicularis oculi startle blink electromyogram (EMG) response (25).



**Fig. 1.** Changes in skin conductance (in microsiemens) resulting from the viewing of threatening and nonthreatening images for high supporters and low supporters of socially protective policies. Difference of means tests: threatening stimuli  $t = 1.98$ ,  $P = 0.05$ ; nonthreatening stimuli  $t = 0.284$ ,  $P = 0.77$ , two-tailed tests. All skin conductance data have been logged. Support for policies is measured by self-reported positions on 18 issues relevant to group life (see text), with “high support” including those participants above the median of support and “low support” including those participants below the median.

<sup>1</sup>Department of Political Science, University of Nebraska-Lincoln, Lincoln, NE 68588, USA. <sup>2</sup>Department of Political Science, Rice University, Houston, TX 77251, USA. <sup>3</sup>Department of Political Science, University of Illinois, Urbana-Champaign, Urbana, IL 61801, USA. <sup>4</sup>Department of Psychology, University of Nebraska-Lincoln, Lincoln, NE 68588, USA. <sup>5</sup>Virginia Institute for Psychiatric and Behavioral Genetics, Richmond, VA 23298, USA.

\*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: jhibbing@unl.edu

Skin conductance “has been closely linked with the psychological concepts of emotion, arousal, and attention” and “provides relatively direct and undiluted representation of sympathetic activity” (26). Arousal causes increased moisture in the outer layers of the skin that in turn enhances conductivity, making it possible to assess sympathetic activation by recording changes in the level of skin conductance. Each participant was shown three separate threatening images (a very large spider on the face of a frightened person, a dazed individual with a bloody face, and an open wound with maggots in it) interspersed among a sequence of 33 images. After logging the data to normalize the distribution, we computed the change in the mean level of skin conductance (SCL) from the previous interstimulus interval (10 s) to the stimulus of interest (20 s). This calculation isolates the change in skin conductance induced by the stimulus and reduces the effects of baseline variations across participants (27). We computed the mean change in SCL induced by the three threatening stimuli and determined whether this mean difference was related to variations in preference for socially protective policies (described below). Similar procedures were conducted for three nonthreatening stimuli shown during the series (a bunny, a bowl of fruit, and a happy child).

The other physiological measure was orbicularis oculi startle blink response, an involuntary response to a startling noise. Harder blinks (higher blink amplitudes) are indicative of a heightened “fear state” (28). The threatening stimulus was a loud, standardized level of white noise heard by participants (through headphones) at seven unexpected moments while they were looking at a computer screen containing nothing but a focus point. As is common practice (28), we first took the logarithm of the data and then computed participants’ average blink amplitude. Because sur-

prising subjects with a sudden, jarring noise is likely to affect all physiological indicators, we conducted the startle portion of the study after completing separate tests on skin conductance. The order of the images and the timing of the auditory startle were randomized once, and then that program was presented to all participants.

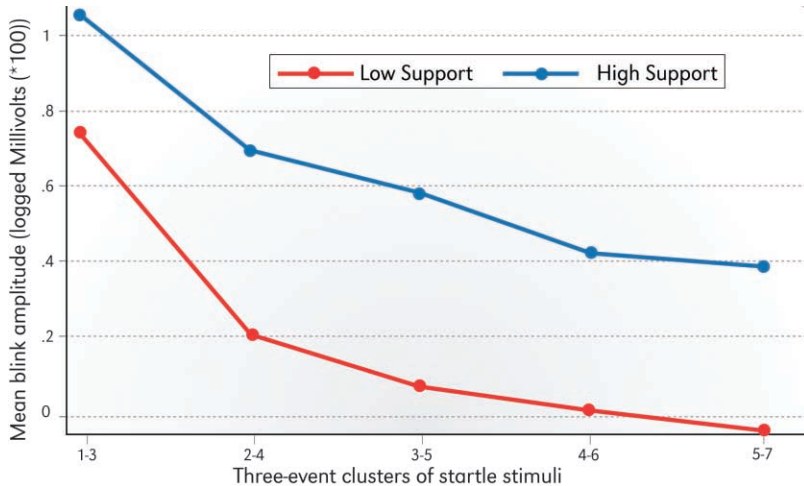
The survey instrument contained a battery of items asking respondents whether they agreed with, disagreed with, or were uncertain toward 28 individual political concepts—the well-known Wilson-Patterson format (29). We identified particular positions on 18 of these policy issues as those most likely to be held by individuals particularly concerned with protecting the interests of the participants’ group, defined as the United States in mid-2007, from threats. These positions are support for military spending, warrantless searches, the death penalty, the Patriot Act, obedience, patriotism, the Iraq War, school prayer, and Biblical truth; and opposition to pacifism, immigration, gun control, foreign aid, compromise, premarital sex, gay marriage, abortion rights, and pornography. We do not label these collections of policy positions as either “liberal” or “conservative” because we measure only one aspect of ideologies and exclude other aspects such as positions on economic issues. We take no stance on whether these positions actually promote the stability and cohesion of the social unit; we only assert that, given the common frames of the modern American policy, those most concerned about social protection will tend to be attracted to the particular policy positions listed.

We computed a summary measure of each participant’s stances on the 18 political issues such that those positions suggesting a concern for protecting the social unit were given higher scores. To test the skin conductance portion of our analysis, we divided participants into two groups according to their level of concern for

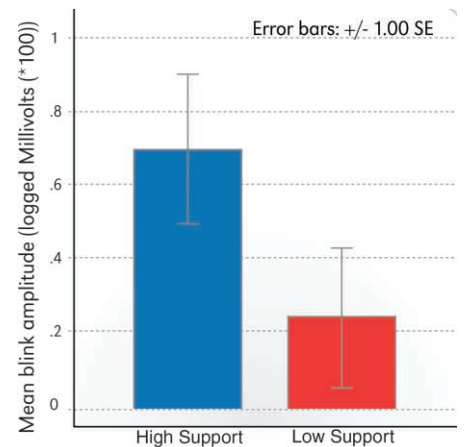
protecting the social unit: those above the median and those below. Participants whose policy positions suggest more concern for protecting the social unit were distinguished by an increase in skin conductance when threatening stimuli were presented (Fig. 1). Those whose positions suggest less concern for protecting the social unit, by contrast, were mostly unaffected by those same stimuli and the difference in these two groups was statistically significant ( $P = 0.05$ ). When participants were shown nonthreatening stimuli, there was no statistically significant difference ( $P = 0.77$ ) in skin conductance changes between the two groups (Fig. 1).

Uncontrolled, bivariate results have the potential to mislead. We therefore regressed each participant’s summary level of support for socially protective political policies on changes in skin conductance as well as on four sociodemographic variables commonly used as predictors of political attitudes: gender, age, income, and education (race and ethnicity were not controlled because all but one participant was self-identified as white and non-Hispanic). With the effects of these sociodemographic variables controlled, the effect of increases in skin conductance when viewing threatening stimuli was positive and significant ( $P < 0.01$ ), with a large standardized regression coefficient (0.377) (Table 1). When nonthreatening images were viewed, however, changes in skin conductance appeared to be unrelated to political attitudes pertaining to protecting the social order. In this multiple regression model, the standardized regression coefficient for skin conductance change was statistically insignificant ( $P = 0.96$ ), small, and slightly negative ( $-0.007$ ) (Table 2).

A further test of this pattern is possible when, for each participant, mean skin conductance change occasioned by the viewing of the nonthreatening stimuli is subtracted from mean skin



**Fig. 2.** Three-event moving average of blink amplitude (in millivolts) in response to seven startling noises administered at unexpected times during the absence of visual stimuli for high supporters and low supporters of socially protective politics. Lines represent mean response for the two groups for each cluster of three responses and are designed to show habituation. All blink amplitude data have been converted to logarithm values so readings less than 0 are possible. Support for policies is as described in Fig. 1.



**Fig. 3.** Mean blink amplitude in response to all seven startling noises for high supporters and low supporters of socially protective politics. Bars are mean blink amplitudes (in millivolts). Difference of means tests for overall means:  $t = 1.64$ ,  $P = 0.10$ . Support for policies is as described in Fig. 1.

conductance change when viewing the threatening stimuli. When this variable was entered into the multiple regression with age, income, education, and gender, it was in the expected direction (greater relative reaction to threatening

stimuli correlates with more support for socially protective policies), sizable (standardized regression coefficient = 0.28), and statistically significant ( $P = 0.04$ ). Full results of this analysis are presented in the SOM.

**Table 1.** Explaining support for socially protective policies with physiological reactions to threatening images. Results of ordinary least squares (OLS) regression with support for socially protective policies (possible range from 0 to 18), with higher numbers indicating attitudes more supportive of policies thought to protect the social unit regressed on five explanatory variables: gender (0 = male; 1 = female), age (in years), education (six categories ranging from "did not finish high school" to "college degree plus"), income (six categories ranging from an annual salary of less than \$20,000 to an annual salary of more than \$100,000), and changes in skin conductance level (SCL) occasioned by the viewing of threatening images. Descriptive statistics on the variables and further discussion of the regression techniques are available in the SOM. \* $P < 0.05$ , two-tailed  $t$  test.

Variable	Unstandardized coefficient (SE)	Standardized coefficient
SCL	92.2* (29.03)	0.377
Income	-0.395 (0.471)	-0.10
Education	-1.63* (0.465)	-0.42
Age	0.19 (0.10)	0.235
Gender	-2.34 (1.3)	-0.20
Constant	-353* (193)	
<i>N</i>	46	
Adj. R-square	0.37	

**Table 2.** Explaining support for socially protective policies with physiological reactions to nonthreatening images. Results of regression (OLS) with support for socially protective policies regressed on five explanatory variables. Variables are the same as those described for Table 1 except that skin conductance (SCL) is the change in skin conductance occasioned by the viewing of nonthreatening images. Descriptive statistics and further discussion of the regression techniques are available in the SOM. \* $P < 0.05$ , two-tailed  $t$  test.

Variable	Unstandardized coefficient (SE)	Standardized coefficient
SCL	-1.8 (35.08)	-0.007
Income	-0.438 (0.533)	-0.115
Education	-1.57* (0.53)	-0.408
Age	0.165 (0.11)	0.204
Gender	-2.23 (1.52)	-0.196
Constant	-304* (217)	
<i>N</i>	46	
Adj. R-square	0.21	

**Table 3.** Explaining support for socially protective policies with blink amplitude in response to startling noises. Results of regression (OLS) with support for socially protective policies regressed on five explanatory variables. Variables are the same as those described for Table 1 except that mean amplitude is the mean blink amplitude for each participant following seven startle events (see Fig. 1). Descriptive statistics and further discussion of the regression techniques are available in the SOM, as is further discussion of the startle technique and measurement procedures. \* $P < 0.05$ , two-tailed  $t$  test.

Variable	Unstandardized coefficient (SE)	Standardized coefficient
Mean amplitude	1.67* (0.75)	0.286
Income	-0.320 (0.500)	-0.08
Education	-1.76* (0.498)	-0.458
Age	-0.187 (0.10)	0.232
Gender	-2.71 (1.45)	-0.239
Constant	-348 (204)	
<i>N</i>	46	
Adj. R-square	0.30	

Startle blink EMG responses habituate (28) (Fig. 2), but the tendency for high blink amplitudes to correlate with respondents supportive of protective policies was consistent across the exercise and was also apparent for the overall means (Fig. 3). Although the difference was not significant in the bivariate analysis, when the sociodemographic controls were added to better specify the model, the coefficient for blink amplitude was again in the predicted (positive) direction, sizable (standardized regression coefficient = 0.286), and statistically significant ( $P = 0.03$ ) (Table 3).

Our data reveal a correlation between physiological responses to threat and political attitudes but do not permit firm conclusions concerning the specific causal processes at work. Particular physiological responses to threat could cause the adoption of certain political attitudes, or the holding of particular political attitudes could cause people to respond in a certain physiological way to environmental threats, but neither of these seems probable. More likely is that physiological responses to generic threats and political attitudes on policies related to protecting the social order may both derive from a common source. Parents could both socialize their children to hold certain political attitudes and condition them to respond in a certain way to threatening stimuli, but conditioning involuntary reflex responses takes immediate and sustained reinforcement and punishment, and it is unlikely that this conditioning varies systematically across political beliefs.

Alternatively, political attitudes and varying physiological responses to threat may both derive from neural activity patterns, perhaps those surrounding the amygdala. There is a connection between localized activation of the amygdala and aversive startle response (30). Amygdala activity is also crucial in shaping responses to socially threatening images (31, 32) and may be connected to political predispositions. Indeed, given that political and social attitudes are heritable (33–36) and that amygdala activity also has been traced to genetics (37–40), genetic variation relevant to amygdala activity could affect both physiological responses to threat and political attitudes bearing on threats to the social order.

Our findings suggest that political attitudes vary with physiological traits linked to divergent manners of experiencing and processing environmental threats. Consequently, our research provides one possible explanation for both the lack of malleability in the beliefs of individuals with strong political convictions and for the associated ubiquity of political conflict.

#### References and Notes

1. A. Campbell, P. E. Converse, W. E. Miller, D. E. Stokes, *The American Voter* (John Wiley, New York, 1960).
2. P. E. Converse, in *Ideology and Discontent*, D. E. Apter, Ed. (Free Press, New York, 1964).
3. J. R. Zaller, *The Nature and Origins of Mass Opinion* (Cambridge Univ. Press, New York, 1992).
4. B. I. Page, R. Y. Shapiro, *The Rational Public* (Univ. of Chicago Press, Chicago, 1992).
5. M. Lodge, C. Taber, *Pol. Psychol.* **26**, 455 (2005).

6. G. E. Marcus, W. R. Neuman, M. Mackuen, *Affective Intelligence and Political Judgment* (Univ. of Chicago Press, Chicago, 2000).
7. R. McDermott, *Perspect. Polit.* **2**, 691 (2004).
8. D. Westen, *The Political Brain* (Public Affairs, New York, 2007).
9. M. J. Landau et al., *Pers. Soc. Psychol. Bull.* **30**, 1136 (2004).
10. S. Fahmy, S. Cho, W. Wanta, Y. Song, *Vis. Commun. Q.* **13**, 3 (2006).
11. J. Faulkner, M. Schaller, J. H. Park, L. A. Duncan, *Group Process. Intergroup Relat.* **7**, 333 (2004).
12. C. D. Navarrete, D. M. T. Fessler, *Evol. Hum. Behav.* **27**, 270 (2006).
13. D. M. Amodio, J. T. Jost, S. L. Master, C. M. Lee, *Nat. Neurosci.* **10**, 1246 (2007).
14. J. T. Jost, J. Glaser, A. W. Kruglanski, F. J. Sulloway, *Psychol. Bull.* **129**, 339 (2003).
15. J. T. Jost, *Am. Psychol.* **61**, 651 (2006).
16. L. Huddy, S. Feldman, C. Taber, G. Lahav, *Am. J. Pol. Sci.* **49**, 593 (2005).
17. S. Feldman, *Pol. Psychol.* **24**, 593 (2003).
18. K. Stenner, *The Authoritarian Dynamic* (Cambridge Univ. Press, New York, 2005).
19. F. Pratto, J. Sidanius, L. M. Stallworth, B. F. Malle, *J. Pers. Soc. Psychol.* **67**, 741 (1994).
20. W. B. Cannon, *Bodily Changes in Pain, Hunger, Fear, and Rage* (Appleton, New York, 1915).
21. M. M. Bradley, P. J. Lang, in *Handbook of Psychophysiology*, J. T. Cacioppo, L. G. Tassinary, G. G. Berntson, Eds. (Cambridge Univ. Press, New York, 2007).
22. M. E. Thase, R. H. Howland, in *Handbook of Depression*, E. E. Beckham and W. R. Leber, Eds. (Guilford, New York, 1995).
23. E. Lemche et al., *Hum. Brain Mapp.* **27**, 623 (2006).
24. G. H. Grosser, H. Wechsler, M. Greenblatt, *The Threat of Impending Disaster* (MIT Press, Cambridge, MA, 1971).
25. Materials and methods are described in the SOM.
26. M. E. Dawson, A. M. Shell, D. L. Filion, in *Handbook of Psychophysiology*, J. T. Cacioppo, L. G. Tassinary, G. G. Berntson, Eds. (Cambridge Univ. Press, New York, 2007).
27. A. Miller, J. Long, in *Developmental Psychophysiology*, L. A. Schmidt, S. J. Segalowitz, Eds. (Cambridge Univ. Press, New York, 2007).
28. P. J. Lang, M. M. Bradley, B. N. Cuthbert, *Psychol. Rev.* **97**, 377 (1990).
29. G. D. Wilson, J. R. Patterson, *Br. J. Soc. Clin. Psychol.* **7**, 264 (1968).
30. S. Anders, M. Lotze, M. Erb, W. Grodd, *Hum. Brain Mapp.* **23**, 200 (2004).
31. C. L. Larson et al., *Biol. Psychiatry* **60**, 410 (2006).
32. D. A. Fitzgerald, M. Angstad, L. M. Jelson, P. J. Nathan, K. L. Phan, *Neuroimage* **30**, 1441 (2006).
33. N. G. Martin et al., *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4364 (1986).
34. L. Eaves et al., *Twin Res.* **2**, 62 (1999).
35. J. R. Alford, C. L. Funk, J. R. Hibbing, *Am. Polit. Sci. Rev.* **99**, 153 (2005).
36. J. H. Fowler, L. A. Baker, C. T. Dawes, *Am. Polit. Sci. Rev.* **102**, 233 (2008).
37. Z. F. Mainen, *Nat. Neurosci.* **10**, 1511 (2007).
38. H. Bracha, D. Yoshioka, N. Masakawa, D. Stockman, *J. Affect. Disord.* **88**, 119 (2005).
39. C. A. Ponder et al., T. C. Gilliam, A. A. Palmer, *Genes Brain Behav.* **6**, 736 (2007).
40. A. R. Hariri et al., *Science* **297**, 400 (2002).
41. We thank E. Whitaker, C. Jacobs, B. Sexton, K. A. Espy, J. Brehm, D. Bulling, and the James Long Company for their invaluable assistance. Financial support was provided by the NSF (SES-0721378 and SES-0721707), the ManTech Corporation, and the University of Nebraska-Lincoln's Strategic Research Cluster Grant program.

#### Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5896/1667/DC1

Materials and Methods

SOM Text

Fig. S1

Tables S1 to S6

Appendix 1

11 March 2008; accepted 18 August 2008

10.1126/science.1157627

# An Alternative Menaquinone Biosynthetic Pathway Operating in Microorganisms

Tomohige Hiratsuka,<sup>1</sup> Kazuo Furihata,<sup>2</sup> Jun Ishikawa,<sup>3</sup> Haruyuki Yamashita,<sup>4</sup> Nobuya Itoh,<sup>1</sup> Haruo Seto,<sup>5</sup> Tohru Dairi<sup>1\*</sup>

In microorganisms, menaquinone is an obligatory component of the electron-transfer pathway. It is derived from chorismate by seven enzymes in *Escherichia coli*. However, a bioinformatic analysis of whole genome sequences has suggested that some microorganisms, including pathogenic species such as *Helicobacter pylori* and *Campylobacter jejuni*, do not have orthologs of the *men* genes, even though they synthesize menaquinone. We deduced the outline of this alternative pathway in a nonpathogenic strain of *Streptomyces* by bioinformatic screening, gene knockouts, shotgun cloning with isolated mutants, and in vitro studies with recombinant enzymes. As humans and commensal intestinal bacteria, including lactobacilli, lack this pathway, it represents an attractive target for the development of chemotherapeutics.

In prokaryotes, ubiquinone and menaquinone (MK) are lipid-soluble molecules that shuttle electrons between the membrane-bound protein complexes in the electron-transport chain (1, 2). For example, the facultative anaerobe *Escherichia coli* uses ubiquinone (CoQ-8) under aerobic conditions but uses MK 8 when it is grown anaerobically. By contrast, many Gram-positive aerobes such as *Bacillus subtilis* contain

only MKs. MK biosynthesis is therefore essential for the survival of these strains. In mammalian cells, ubiquinone plays a role in the electron-transport chain in the inner mitochondrial membrane, and MK functions as an essential vitamin for the biological activation of a family of proteins involved in blood coagulation (3), bone metabolism (4), and cell-cycle regulation (5). The biosynthesis of MK had been mainly studied in *E. coli*. In this organism, chorismate, which is derived from the shikimate pathway, is converted into MK by seven enzymes (MenA to MenG, Fig. 1). Although humans lack this pathway, essential amounts of MK are normally supplied in the diet.

There is no trace of *menF*, *menD*, *menC*, *menE*, and *menB* gene orthologs in the genome of *Streptomyces coelicolor* A3(2) (6–8), even though it produces MKs. Similarly, some pathogens that synthesize MK, including *Helicobacter*

*pylori* and *Campylobacter jejuni*, have also been reported to lack *men* gene homologs (9–12). We performed a tracer experiment with *S. coelicolor* A3(2) and [U-<sup>13</sup>C<sub>6</sub>]glucose to test whether an alternative pathway to MK operated in the strain. We found that the labeling patterns of MK differed from those of the classical pathway and that 1,4-naphthoquinone-6-carboxylic acid (Fig. 1) (or its reduced form, 1,4-dihydroxy-6-naphthoate) was an intermediate, which suggests that MK is indeed biosynthesized by an alternative route in this species (13). We have identified the genes, enzymes, and biosynthetic intermediates responsible for this alternative pathway, which we have named the futasoline pathway.

We started our investigations by screening genome databases, as well as mutants that require MK for growth. We found that some microorganisms in the epsilon categories of Proteobacteria, Actinobacteria, and the *Deinococcus-Thermus* bacteria groups lacked *men* gene orthologs despite the fact that most of these strains are known to synthesize MKs. From among these, we selected four microorganisms for further analysis: *H. pylori*, *C. jejuni*, *Thermus thermophilus* (14) and *S. coelicolor*. We also made comparisons with microorganisms in which the known MK biosynthetic pathway operates, including *E. coli* (15), *Bacillus subtilis* (16), *Corynebacterium glutamicum* (17), and *Mycobacterium tuberculosis* (18). To find candidate genes, we first estimated orthologous genes as reciprocal best-hit pairs using the BLAST (Basic Local Alignment Search Tool) program (19) with a cutoff *e* value < 10<sup>-10</sup> and then searched for candidate genes present in the *men* negative group but absent in the *men* positive group. We eventually identified ~50 candidate genes in *S. coelicolor* A3(2). Putative transcriptional regulators and membrane proteins such as adenosine triphosphate-binding cassette transporters that are known to transport a

<sup>1</sup>Biotechnology Research Center, Toyama Prefectural University, Toyama 939-0398, Japan. <sup>2</sup>Division of Agriculture and Agricultural Life Science, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657 Japan. <sup>3</sup>Department of Bioactive Molecules, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo 162-8640, Japan. <sup>4</sup>Advanced Materials Research and Development Laboratory, ADEKA Corporation, Arakawa-ku, Tokyo 116-8553, Japan. <sup>5</sup>Faculty of Applied Bioscience, Tokyo University of Agriculture, Setagaya-ku, Tokyo 156-8502, Japan.

\*To whom correspondence should be addressed. E-mail: dairi@pu-toyama.ac.jp