The published structures of the transition-state-analogue complexes of RhoGDP.AlF_4^-/p50rhoGAP (ref. 5) and RasGDP.AlF_3/p120rasGAP (ref. 6) show that the two GAP domains bind their respective G proteins in related but distinct ways. rhoGAP binds to Rho primarily through a surface made up by the helices B and F, whereas rasGAP binds to Ras through the antiparallel helices 6c and 7c.

These differences in G-protein-binding surfaces arise because of the different spatial relationship between helices A–B and E–F of rhoGAP and 1c–2c and 5c–6c of rasGAP. Relative to the rhoGAP helices E and F, the carboxy terminal (or top half) of 5c bends back into the plane of the page while the whole of 6c rotates further in a similar direction.

Nonetheless, both GAP domains present their catalytic arginine residues to the active sites of the G protein in very similar ways, which is a consequence of the equivalence in function of the arginine loop in these two systems. It will be intriguing to see whether other GAPs associated with the Ras superfamily contain the same core structure, obey the same topology, and make similar use of hydrophobic clamping to orient the catalytic arginine loop.

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Meningitis bacterium is viable without endotoxin

The outer membrane of Gram-negative bacteria contains lipopolysaccharide (LPS) as its outer monolayer. This is anchored to the membrane by lipid A, which is responsible for LPS's activity as an endotoxin[1]. In *Escherichia coli*, conditionally lethal mutants have been reported for the genes involved in the early steps of lipid A biosynthesis[2], suggesting that this part of the LPS molecule is essential for bacterial growth. However, we have isolated a mutant of *Neisseria meningitidis* which is viable in spite of an early block in lipid A biosynthesis that causes a loss of endotoxin activity.

The protein LpxA is responsible for adding the *O*-linked 3-OH fatty acid to UDP–*N*-acetylglucosamine, which is the first committed step in the lipid A biosynthesis pathway[3,4]. The *E. coli* and *N. meningitidis* LpxA proteins differ in their specificity, preferring 3-OH C_{14} and 3-OH C_{12} fatty acyl chains, respectively[5,6].

We constructed a hybrid *lpxA* gene in which the meningococcal N-terminal part was replaced by the corresponding part of *E. coli lpxA*. Plasmid pHBK30, carrying this hybrid gene and an upstream kanamycin-resistance cassette, was used for allelic replacement of the wild-type *lpxA* gene on the chromosome of meningococcal strain H44/76. Lipopolysaccharide of the H44/76[pHBK30] mutant and the wild-type strain was compared by Tricine-SDS–PAGE followed by silver staining for carbohydrates (Fig. 1a). No LPS could be detected in the hybrid derivative by this method, even when higher amounts of cell lysates were loaded on the gel.

A panel of LPS and monoclonal antibodies specific for outer-membrane proteins (OMPs) was tested in a whole-cell enzyme-linked immunosorbent assay (ELISA)[7,8]. The mutant strain did not bind any of the LPS-specific antibodies (either immunotype-specific or broadly crossreactive), whereas the OMP-specific antibodies showed similar binding patterns for mutant and wild type. This apparent OMP similarity was confirmed when outer-membrane complexes (OMCs) of H44/76[pHBK30] and H44/76 were isolated by sarkosyl extraction and analysed by SDS–PAGE (Fig. 1b). Both strains show equal amounts of the class 1, 3 and 4 principal OMPs.

As LPS of H44/76[pHBK30] could not be detected, it was not clear whether it was present at all. Therefore, the mutant and wild-type strain were tested in a chromogenic *Limulus* (LAL) assay[9] for endotoxin, using the QCL-1000 kit from BioWhittaker. The results of the LAL assay on cell suspensions showed no significant endotoxin activity for H44/76[pHBK30] over meningococcal medium (0.3 and 1.7 endotoxin units per millilitre, respectively), in contrast to 21.7×10^4 endotoxin units per millilitre for the wild type.

Although H44/76[pHBK30] is viable, it had a reduced growth rate. When grown overnight on GC agar plates, the mutant strain produced much smaller colonies. In liquid medium, the doubling time during exponential growth was about 50% longer than in the wild-type strain H44/76. The morphology of H44/76[pHBK30] and its parent strain was examined by electron microscopy of ultrathin sections (Fig. 1c). The ultrastructure of the outer membrane could be clearly discerned in the LPS-deficient mutant and was not visibly altered.

Strain H44/76[pHBK30] is viable and possesses an outer membrane, despite lacking detectable LPS. It seems likely that the hybrid *lpxA* gene is inactive, either because of disrupted transcription/translation in our construct, or otherwise because the hybrid protein produced lacks enzymatic activity. We constructed an *lpxA*-knockout mutant by inserting a kanamycin-resistance cassette into the *Bst*EII site located at position 293 in the *lpxA* gene of plasmid pLA21 (a pUC18 derivative with a 2.1 kilobase *lpxD-fabZ-lpxA* insert). The resulting plasmid pLAK33 was linearized and transformed to strain H44/76, with selection for kanamycin resistance. The resulting colonies showed the same growth properties as the H44/76[pHBK30] mutant. In whole-cell ELISA, the *lpxA*-knockout mutant did not bind any of the LPS-specific monoclonal antibodies.

The absence of LPS was further confirmed by gas chromatography/mass spectrometry analysis of fatty acids present in OMC preparations, which showed that the lipid A-specific 3-OH C_{12}, whose addition

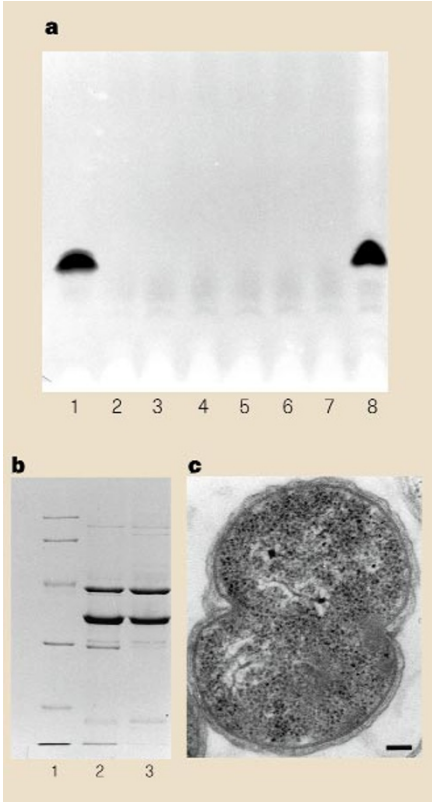

Figure 1 Analysis of *Neisseria meningitidis* outer membrane. **a,** Silver-stained Tricine-SDS–PAGE LPS gel of proteinase K-treated whole-cell lysates of H44/76 wild type (lanes 1 and 8) and six independent kanamycin-resistant transformants with pHBK30 (lanes 2–7). **b,** SDS–PAGE of outer membrane proteins from H44/76[pHBK30] (lane 2) and H44/76 wild type (lane 3); lane 1 contains relative molecular mass markers of 94K, 67K, 43K, 30K, 20.1K and 14.4K. **c,** Electron micrograph of a thin section of H44/76[pHBK30], showing normal appearance of the cell envelope with outer membrane, peptidoglycan layer within the periplasmic space, and inner membrane. Scale bar, 100 nm.

to UDP–*N*-acetylglucosamine is catalysed by LpxA, was present only in trace amounts in the mutant. Also, no other *O*-linked 3-OH fatty acids in the range C_{10} to C_{20} could be detected. LPS biosynthesis could be restored in this knockout mutant by retransformation with wild-type *lpxA*, showing that the observed LPS deficiency must result from mutation in this gene only.

The availability of LPS-deficient mutants will allow new approaches to vaccine development against *N. meningitidis*[10] and the closely related pathogen *N. gonorrhoeae*, or any other bacteria for which such mutants can be isolated. Using an *lpxA* mutant, it will be much easier to purify OMPs or other cell-surface components without contamination by endotoxin. Also, the role of LPS in outer-membrane-vesicle or whole-cell vaccines (for example as adjuvant[11]) can be investigated: it may be possible to substitute a less toxic compound. And at a more basic level, such mutants can be used for studying how LPS contributes to the structure and biogenesis of the outer membrane.

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Speed perception fogs up as visibility drops

Many horrendous vehicle accidents occur in foggy weather. Drivers know they should slow down because fog reduces visibility, but many still drive too quickly[1]. The 'blame' for many such accidents may be due to a perceptual quirk: it appears that drivers think they are driving far more slowly than they actually are in foggy conditions, and therefore increase their speed.

We used a virtual-environment driving simulator to show that, as fog increases and therefore reduces the contrast of the driver's image, the apparent speed of the vehicle slows. Participants asked to drive at a certain speed drove faster as the scene became foggier.

An accurate representation of the current speed is normally provided to drivers by the speedometer. However, reading this instrument requires drivers to divert their gaze and attention from the road to the appropriate dial. In conditions of reduced visibility produced by fog, drivers are reluctant to divert their gaze from the road to the speedometer for fear of missing an object emerging from the fog[2]. Hence it is exactly in conditions of reduced visibility caused by fog that drivers rely on their own perceptual judgement of speed.

Thompson[3] has reported that the perceived speed of a moving-grating pattern depends on its level of contrast. As contrast decreases, the grating appears to drift more slowly. This effect has been replicated several times (and extended to other stimulus patterns[4]), but others have failed to replicate it or have shown that perceived speed is unaffected by random variations in contrast[5]. So it remains an open question as to whether this illusion of reduced speed with decreasing image-contrast will occur in conditions akin to those encountered when driving in fog.

We tested for the perceived slowing of a visual scene by conducting two experiments in a virtual environment that simulated the view from a vehicle moving along a road. Such a set-up allowed us to manipulate the visual stimulus in a highly specific manner.

Our first experiment involved showing the observer two scenes. In one scene, the weather was 'clear'; in the other, it was either 'clear', 'misty' or 'foggy'. The physical speed at which the two scenes appeared to move at the same speed was calculated (Fig. 1a) using standard psychophysical techniques. Subjects perceived the foggier scenes to be moving more slowly[6].

Does this perceptual change affect driving speed in a more realistic task? We trained subjects (in clear conditions) to 'drive' a simulated vehicle at set speeds along a winding road, using a brake, accelerator and steering column.

In the experimental phase, the subject was given a target speed and told to adjust the car speed (while steering along the road) to this target, using the accelerator and brake. Subjects accelerated and decelerated as they thought appropriate for as long as they pleased, and then signalled that they believed that they were travelling at the target speed. Trials in which the weather was 'clear', 'misty' and 'foggy' were randomly interleaved. As the scene became more foggy, subjects drove at faster speeds (Fig. 1b).

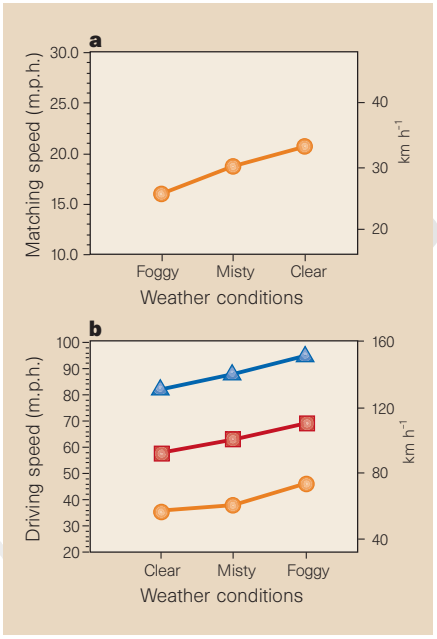

Figure 1 Sense of speed decreases in fog. **a,** Perceived matching speed averaged over all subjects (n = 5) in three weather conditions under passive viewing. Foggier scenes gave the impression of slower movement (one-way analysis of variance; $P < 0.001$). Simulation was performed using a Silicon Graphics Crimson Reality Engine that updated the viewing screen (60 Hz VDU monitor) at a rate of 15 Hz. 'Fog' was simulated by blending a partially transparent polygon over each pixel. For each pixel, the red, green and blue guns levels were recalculated (R') from the original pixel values (R) drawn from memory according to the formula, $R' = 255F + (1 - F)R$. 'Clear' conditions: $F = 0$, contrast (root mean square) = 0.61; 'misty': $F = 0.5$, contrast = 0.20; 'foggy': $F = 0.8$, contrast = 0.07. **b,** The actual speed driven under three weather conditions (n = 9). Target speeds: circles, 30 m.p.h. (48 km h^{-1}); squares, 50 m.p.h. (80 km h^{-1}); triangles, 70 m.p.h. (112 km h^{-1}). Analysis of variance showed significant effects of target speed ($P < 0.0001$) and weather conditions ($P < 0.0001$), but no interaction ($F < 1$).

This finding suggests that the 'blame' for many such accidents may not lie solely in the irresponsible nature of the drivers but with an unfortunate quirk of our perceptual systems.

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