Response by Howard Wolinsky

The correspondence from Anna Olsson and Augusto Vitale provides some perspective that is indeed missing from our October 2009 article. I will not disagree with them, although I would like to point out that this lack of perspective comes not as a result of our own work but from the fact that some material had to be omitted just before the article went to press. I thus feel compelled to explain the story behind the story.

The article started out as a look at how the animal rights movement is affecting policy in the USA and Europe. However, as we proceeded with the story we noted pending legislation on both continents and shifted the focus of the piece to include this. We carefully selected sources on both sides of the Atlantic in an effort to provide informative voices with varying, balanced views. We were especially intrigued by the pending revision of the European Union's Directive 86/609.

I was lucky enough to contact a desk officer at the European Commission (EC) in Brussels who was involved with the revision. She consented to a taped interview over the telephone with the understanding that she would be able to review our use of her answers—the editor at *EMBO reports* agreed to this.

For the record, journalism as practiced in the USA, where we work, can be different from that practiced in Europe and elsewhere. Different rules might also apply if you're writing for a daily newspaper, a trade publication or a science publication. US newspaper editors do not generally want their reporters to have their work reviewed by the people about whom they are reporting; especially if those people are politicians. Conversely, some, perhaps many science journalists these days do ask their sources to review articles in the interest of avoiding errors.

When a bureaucrat in the USA or Europe asks to review an article before it goes to press, it therefore goes against my instinct and training. But I went along with such a review—with the blessing and caveats from the editor at *EMBO reports*—to get the interview.

The bureaucrat agreed to speak on record. As per her request, I even sent ahead preliminary questions. The official wrote back that the EC would wish to see and approve our article before publication. That should have been a warning of troubles ahead. Still, we gained a great deal of insight from the interview into the Commission's thinking concerning the Directive, how the laws were likely to change and some of the behind-the-scenes politics. We included all this information in our original article.

After the interview, the official went on vacation and returned just as our deadline approached. We sent her the article for review and she sent it to her communications department, which refused to grant permission to use any of the information we gained from her; they pointed out no inaccuracies. Their response broke our agreement and their refusal came right on the production deadline.

The story had a hole. We debated different ways of how to address the problem, but felt ultimately that we couldn't paraphrase the interview; nor did we feel comfortable attributing quotes to a nameless EC bureaucrat. Time was up. The story was set in type and had to run with the EC declining to comment. As we removed the quotes and information from the interview, the article inevitably shifted in balance to become more critical of the proposed changes to European animal research legislation. Needless to say, it was too late to identify and interview other sources who could have provided the now-missing balance.

Over the years, I have had several successful experiences dealing with the EC. This was not one of those. Olsson and Vitale now make some of the same points that the EC official made concerning the new legislation; in that sense, the system works. In public policy, the media, elected officials, bureaucrats and the various publics all have important roles. But this is a fragile ecosystem that breaks down when officialdom opts out. This can create the impression of bias where none exists, or worse, it can cause the various constituencies, including the public and scientists, to lose out when information does not flow freely in spirited debate. I have the sense that bureaucrats are centred on job security in general and do not want to rock the boat. The authorship of a favourite quote is in dispute. But to paraphrase, there are two things you shouldn't see made: laws and sausage. My version is: laws, sausage and occasionally news coverage of laws in Brussels and Washington in the absence of transparency.

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Helicobacter pylori induces NF-κB independent of CagA

We refer to the publication by Lamb *et al* (2009). This report describes the *Helicobacter pylori* virulence factor cytotoxin-associated gene A (CagA), encoded in the pathogenicity island (PAI), as essential for the rapid activation of the transcription factor nuclear factor κB (NF-κB) in epithelial cells. The authors reported fast NF-κB activation in AGS (human gastric carcinoma) cells infected with *H. pylori* G27 wild type, but not with the isogenic CagA-deficient strain, determined by IκBα phosphorylation and degradation, RelA phosphorylation, NF-κB DNA-binding, and interleukin 8 (IL-8) mRNA expression.

However, the profound CagA dependency of NF-κB activation, representing the centrepiece of the study by Lamb *et al* (2009), is in clear contradiction to previously published data including data from our laboratory. Early documentation in the literature indicates type IV secretion system/PAI-dependent—but CagA-independent—rapid activation of NF-κB by *H. pylori* in epithelial cells, determined by NF-κB DNA-binding, NF-κB transactivation activity or IL-8 secretion (for example: Sharma *et al* 1995; Censini *et al* 1996; Fischer *et al* 2001; Neu *et al* 2002; Foryst-Ludwig *et al* 2004). For more than 10 years, our lab has routinely monitored NF-κB activation in *H. pylori*-infected cells, and we find that IκBα phosphorylation and degradation, RelA phosphorylation (Fig 1), DNA-binding and induction of IL-8 are reproducibly induced by *H. pylori* wild-type and isogenic CagA-deficient strains to an almost equal extent. A slight delay in the induction of NF-κB by the CagA-deficient strain can be explained, for example, by its slightly delayed attachment to host cells.

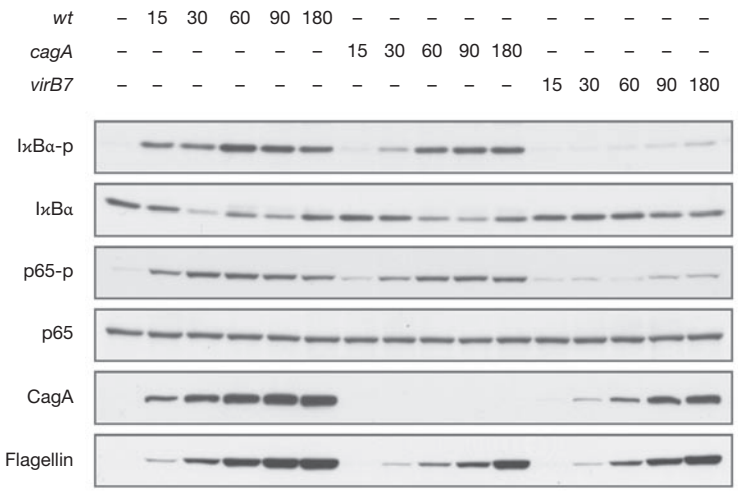

Fig 1 | Rapid NF-κB activation by *Helicobacter pylori* is type IV secretion system (T4SS)-dependent and CagA-independent. AGS cells were infected (MOI of 100) with the P1 *H. pylori* wild-type (*wt*) strain, the CagA-deficient (*cagA*) or T4SS-deficient (*virB7*) isogenic mutant strains. At the indicated times after infection, whole cell lysates were prepared and analysed by SDS-PAGE and western blotting. For immunodetection, protein-specific or phosphoprotein-specific antibodies (phospho-IκBα Ser32/36; phospho-p65 Ser536) were used, as indicated.

In our opinion, a single CagA-deficient *H. pylori* strain that is able to rapidly activate NF-κB with a potency similar to the wild-type strain represents strong evidence against an essential requirement for CagA, irrespective of the genetic background of the strain. Although few studies suggest a contributory role of CagA in late *H. pylori*-induced NF-κB activation (for example, Suzuki *et al* 2009), these studies address long-term effects involving indirect processes, but not direct NF-κB activation within the first hour of infection.

As a possible explanation for the conflicting data, we would like to refer to the publication by Fischer *et al* (2001). Therein, the authors stated that great care needs to be taken in the generation and propagation of *H. pylori* isogenic mutant strains to prevent accidental functional inactivation of the PAI concomitant with the deletion of single genes encoded in it: a phenomenon named 'polar effect'. Functional inactivation of the PAI due to the 'polar effect' has been documented in the literature (Naumann *et al* 1999). Thus, complementation of the *cagA*-deficient *H. pylori* strains with *cagA* could have unequivocally proven the suggested function of CagA in NF-κB activation.

We think our comment offers fair, putative reasons for the conflicting data and helps to diminish uncertainty in the scientific community.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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Response: strain-specific activation of NF-κB by *Helicobacter pylori* CagA

We appreciate the comments from Schweitzer *et al* in this issue about our discovery that *Helicobacter pylori* cytotoxin-associated gene A (CagA) activates nuclear factor κB (NF-κB), as they reveal the lack of consensus on the role that CagA plays during *H. pylori* infection.

We are confident in our data for several reasons. First, we demonstrated in our studies that three independent *H. pylori* *cagA*-positive strains, but not their respective isogenic *cagA* mutants, activate NF-κB. Second, because *cagA* is monocistronic and transcribed in a different direction than other genes comprising the *cag* pathogenicity island (*cag*PAI; Censini *et al*, 1996), it is unlikely that our *H. pylori* isogenic *cagA* mutant strains are exerting "polar effects" on other genes within the *cag*PAI. Third, these strains have been used extensively by other groups without apparent evidence of potential polar effects due to disruption of *cagA* (for example, Brandt *et al*, 2005; Franco *et al*, 2005; Guillemin *et al*, 2002). Fourth, while many studies demonstrate the importance of the *cag*PAI-encoded type 4 secretion system (T4SS) in *H. pylori*-induced pathogenesis and inflammation (Glocker *et al*, 1998), CagA is injected into host epithelial cells via the T4SS (Odenbreit *et al*, 2000), and studies that show defects in the ability of T4SS-deficient bacteria to activate NF-κB cannot completely exclude the importance of CagA. Lastly, the demonstration that CagA is important for the activation of NF-κB is not entirely unprecedented. Using an interleukin (IL) 8 promoter-reporter assay, Sharma *et al* (1998) showed the requirement of CagA for NF-κB activation. Brandt *et al* (2005) demonstrated that ectopically expressed CagA induces the translocation of NF-κB from the cytoplasm into the nucleus, and, in accord with a study from Kim *et al* (2006), showed that ectopic CagA expression induces IL-8 secretion from gastric epithelial cells. Furthermore, Shibata *et al* found that Mongolian gerbils infected with *cagA*-deficient *H. pylori* showed significantly decreased NF-κB activation and NF-κB-linked inflammation in the gastric antra of infected gerbils (2006). These data,