In this issue of *Cell*, Wei et al. (2006) demonstrate that the low-density lipoprotein receptor-related protein 6 (LRP6) promotes endocytosis of the anthrax toxin into cells. LRP6 act as a coreceptor with either TEM8 or CMG2, the two previously identified receptors for anthrax toxin.

Although not considered among the usual suspects in community and hospital acquired microbial infections, the public eye turned to *Bacillus anthracis*, a Gram-positive bacterium, after the anthrax attacks in October of 2001. Ever since, it has become clear that *Bacillus anthracis* and its potent toxin pose a serious threat to human health when exploited as a weapon of biological warfare. Measures must be in place to deal with a true anthrax outbreak and its consequences, which undoubtedly would include the emergence of strains resistant to current drugs of choice, such as ciprofloxin (better known by its brand name Cipro).

The anthrax toxin is secreted by vegetative anthrax and is comprised of three separate proteins: lethal factor (LF), a metalloproteinase that cleaves mitogen-activated kinase kinases; edema factor (EF), a calcium-calmodulin-dependent adenylyl cyclase; and the protective antigen (PA), the protein responsible for the import of the toxin into cells and its ultimate release into the cytosol (Collier and Young, 2003). The initial recognition between PA and the host receptors, ATR/tumor endothelial marker 8 (TEM8) and/or capillary morphogenesis protein 2 (CMG2), is critical for entry of the toxin (Scobie and Young, 2005). Binding of PA to the receptor leads to cleavage of PA by furin, and the subsequent formation of an oligomeric structure of PA proteins called the prepore (Figure 1). This allows binding of EF or LF to the prepore, and after this, endocytosis occurs. In the endosomal compartment, following internalization, LF and EF reach the cytosol through a pH-dependent conversion of the prepore to a pore. Mechanistically, the complex of LF and the prepore on the cell surface promote formation

![Figure 1. Model for Anthrax Toxin Endocytosis](image-url)

*The receptors for anthrax toxin CMG2/TEM8 (purple) form a complex with LRP6 (yellow) through the extracellular domains of both proteins. Upon binding of PA$_{63}$, a possible conformational change (depicted as the conversion of a rectangle to an oval) occurs in LRP6 that is propagated to the C-terminal cytoplasmic domain. Furin cleavage leads to the release of PA$_{20}$ and the formation of a heptameric pore precursor. Binding of lethal factor (LF) or edema factor (EF) to the pore precursor is followed by endocytosis, which is known to be a clathrin-dependent process. It is currently unknown at which point after binding to the receptor the internalization signal from LRP6 is transferred to another protein (perhaps GSK-3), but the transfer likely follows heptamer formation or the binding of LF/EF.*
of lipid rafts and clathrin-dependent endocytosis (Liu and Leppa, 2003), a process that has been shown to sequester the low-density lipoprotein receptor (Brown and Goldstein, 1979). Because PA binding to the CMG2 receptor is not sufficient for endocytosis of the toxin into cells (Abrami et al., 2003), questions remained regarding the existence of other factors critical for endocytosis of the toxin following the initial liaison between the toxin and the preprepore. In discovering that the low-density lipoprotein receptor-related protein 6 (LRP6) is required for anthrax toxin endocytosis, Cohen and coworkers (Wei et al., 2006) have now provided a critical piece to the puzzle.

The authors identified LRP6 as a new protein important for anthrax toxicity through a creative approach to screen and identify genes that are important for entry of the toxin into cells. A library of ~40,000 antisense ESTs (expressed sequence tags) was introduced into a population of cells using a lentivirus. These ESTs were able to bind to mRNA and inhibit their translation into protein. These cells were treated with PA along with a modified form of lethal factor, called FP59. Cells resistant to the combination of PA and FP59 were isolated, and one clone, ATR43, showed particular resistance to PA. Isolation of the plasmid expressing the EST and subsequent sequenc- ing showed that it encoded lrp6.

LRP6 is not a stranger to the cell biologist. LRP6 is a single-span transmembrane protein and is a key component of the Wnt signaling pathway, which regulates the level of β-catenin levels in the cell and is important in development and proliferation. Recent work has shown that Wnt directly binds to LRP6 and activates both casein kinase I and GSK-3β activity, which in turn phosphorylates LRP6 in the C-terminal domain and facilitates binding to the scaffold protein Axin. Ultimately, this prevents the degradation of β-catenin by the proteosome and promotes formation of the dorsal-ventral axis in embryogenesis (Zeng et al., 2005).

It should be noted that the authors of the current report isolated several clones in their screen that are resistant to PA/FP59 toxicity, many of which have yet to be identified. Similarly, previous work has shown that cells lacking ARAP3, a phosphoinositide binding protein, are particularly resistant to anthrax toxicity. ARAP3 mediates phosphoinositide-3 (PI3)-kinase rearrangements in cells and functions to regulate the activity of the GTPase Arf6 (Lu et al., 2004). Clearly, ARAP3 is also important for toxicity, but how this relates to LRP6 is not yet understood.

Several other lines of evidence in this elegant study support the idea that LRP6 is an accessory protein to toxin internalization. First, the use of antisense expression of the lrp6 EST in naïve cells (that is, not treated with the lentivirus) blocked expression of lrp6 and generated toxin resistance. Second, immunoprecipitation showed that LRP6 directly interacts with the receptors TEM8 and CMG2. Third, macrophages, which are a major target for LF-induced lethality, were also protected from PA/LF when a specific short interfering RNA (siRNA) corresponding to the antisense strand of lrp6 was expressed. Perhaps most importantly, from the standpoint of efforts to develop drugs that block anthrax infection, antibodies directed against the extracellular domain of LRP6 provide protection against PA/LF-induced lethality.

Wei et al. (2006) suggest a model consistent with these data in which the extracellular domain of LRP6 initially binds to the extracellular domains of either CMG2 or TEM8 (Figure 1). Upon binding to the PA/LF or EF complex, furin cleavage of PA occurs, leading to the formation of a heptameric complex that is the precursor of the pore. The authors propose that the signal for internalization is mediated through the extracellular domain of LRP6 and is propagated to its intracellular C-terminal domain. It is not propagated through the C-terminal domain of CMG2; removal of this domain does not affect the entry of anthrax toxin into cells (Liu and Leppa, 2003). How is this signal from LRP6 propagated? Wei et al. (2006) provide evidence that endocytosis may not be dependent on the Wnt signaling pathway because disruption of Wnt signaling by overexpression of Axin failed to provide resistance to PA/FP59 toxicity. However, exposure of macrophages to the anthrax toxin also results in decreased levels of GSK-3, suggesting that GSK-3 is a point shared by both the Wnt and the ATR/TEM8/CMG2 signaling pathways (Tucker et al., 2003). More work is needed to delineate how these three pathways converge (and diverge). Like LRP6, the CMG2 receptor is found in a wide variety of tissue types and has been implicated in influencing basement membrane assembly through interactions with collagen IV and laminin (Bell et al., 2001). However, despite the extensive characterization of LRP6, the biological function of CMG2 remains elusive. Perhaps this study will provide a much needed impetus toward defining the function of CMG2 (along with LRP6) not only as a toxin receptor but also as a probable regulator of the cell cycle and cell proliferation.

Thus, Wei and coworkers have intercepted critical lines of dialog in the conversation between Bacillus anthracis and its mammalian host, which may lead to the development of countermeasures to anthrax infectivity that are based on blocking interactions between the toxin receptors and LRP6. It was nearly 10 years ago that Cell reported that the chemokine receptor CKR5 (CCR5) was missing from a population of individuals who were seemingly resistant to HIV-1, despite multiple exposures to the virus (Liu et al., 1996). Since then, understanding how the complex of CD4, the receptor for HIV, and CCR5, the coreceptor, modulates the entry of HIV-1 has been a focus of AIDS research and has lead to the development of therapeutics designed to block the interaction between CCR5 and CD4.
As with the identifica-
tion of CCR5 and its influence on
AIDS research, we may look back
in 10 years on this discovery as a
major breakthrough in understand-
ing anthrax pathogenesis.

REFERENCES

Abrami, L., Liu, S., Cosson, P., Leppla, S.H.,
160, 321–328.

Bell, S.E., Mavila, A., Salazar, R., Bayless,
K.J., Kanagala, S., Maxwell, S.A., and Davis,

Natl. Acad. Sci. USA 76, 3330–3337.


278, 5227–5234.

Liu, R., Paxton, W.A., Choe, S., Ceradini, D.,
Martin, S.R., Horuk, R., MacDonald, M.E.,

Lu, Q., Wei, W., Kowalski, P.E., Chang, A.C.Y.,
USA 101, 17246–17251.


Opin. Microbiol. 8, 106–112.

Tucker, A.E., Salles, I.I., Voth, D.E., Ortiz-
Leduc, W., Wang, H., Dozmozov, I., Centola,
5, 523–532.

Wei, W., Lu, Q., Chaudry, G.J., Leppla, S.H.,

Zeng, X., Tamai, K., Doble, B., Li, S., Huang,
H., Habas, R., Okamura, H., Woodgett, J., and