Systematic genetic nomenclature for type VII secretion systems

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

| Citation          | Bitter, Wilbert, Edith N. G. Houben, Daria Bottai, Priscille Brodin, Eric J. Brown, Jeffery S. Cox, Keith Derbyshire, et al. 2009. Systematic genetic nomenclature for type VII secretion systems. PLoS Pathogens 5(10): e1000507. |
|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Published Version | doi:10.1371/journal.ppat.1000507                                                                                                                                                                                                                                                                                  |
| Citable link      | http://nrs.harvard.edu/urn-3:HUL.InstRepos:4879205                                                                                                                                                                                                                                                                  |
| Terms of Use      | This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA                                                                                                                                 |

Systematic Genetic Nomenclature for Type VII Secretion Systems

Wilbert Bitter¹*, Edith N. G. Houben¹, Daria Bottai², Priscille Brodin³, Eric J. Brown⁴, Jeffery S. Cox⁵, Keith Derbyshire⁶, Sarah M. Fortune⁷, Lian-Yong Gao⁸, Jun Liu⁹, Nicolaas C. Gey van Pittius¹⁰, Alexander S. Pym¹¹, Eric J. Rubin¹², David R. Sherman¹², Stewart T. Cole¹³, Roland Brosch¹⁴*

¹ VU University Medical Centre, Amsterdam, The Netherlands, ² Dipartimento di Patologia Sperimentale, Biotecnologie Mediche, Infettivologia ed Epidemiologia, University of Pisa, Pisa, Italy, ³ Inserm Avenir Group, Institut Pasteur Korea, Seoul, Korea, ⁴ Department of Microbial Pathogenesis, Genentech Inc., San Francisco, California, United States of America, ⁵ Department of Microbiology and Immunology, University of California, San Francisco, California, United States of America, ⁶ Wadsworth Center, New York State Department of Health, Albany, New York, United States of America, ⁷ Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts, United States of America, ⁸ Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland, United States of America, ⁹ Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada, ¹⁰ Department of Biomedical Sciences, Stellenbosch University, Stellenbosch, South Africa, ¹¹ Unit for Clinical and Biomedical TB Research, South African MRC, Durban, South Africa, ¹² Seattle Biomedical Research Institute, Seattle, Washington, United States of America, ¹³ Global Health Institute, EPFL, Lausanne, Switzerland, ¹⁴ Institut Pasteur, Integrated Mycobacterial Pathogenomics, Paris, France

Myobacteria, such as the etiological agent of human tuberculosis, Mycobacterium tuberculosis, are protected by an impermeable cell envelope composed of an inner cytoplasmic membrane, a peptidoglycan layer, an arabinogalactan layer, and an outer membrane. This second membrane consists of covalently linked, tightly packed long-chain mycolic acids [1,2] and non-covalently bound shorter lipids involved in pathogenicity [3–5]. To ensure protein transport across this complex cell envelope, mycobacteria use various secretion pathways, such as the SecA1-mediated general secretory pathway [6,7], an alternative SecA2-operated pathway [8], a twin-arginine translocation system [9,10], and a specialized secretion pathway variously named ESAT-6, SNM-, ESX-, or type VII secretion [11–16]. The latter pathway, hereafter referred to as type VII secretion (T7S), has recently become a large and competitive research topic that is closely linked to studies of host–pathogen interactions of M. tuberculosis [17] and other pathogenic mycobacteria [16]. Molecular details are just beginning to be revealed [18–22] showing that T7S systems are complex machineries with multiple components and multiple substrates. Despite their biological importance, there has been a lack of a clear naming policy for the components and substrates of these systems. As there are multiple paralogous T7S systems within the Mycobacteria and orthologous systems in related bacteria, we are concerned that, without a unified nomenclature system, a multitude of redundant and obscure gene names will be used that will inevitably lead to confusion and hinder future progress. In this opinion piece we will therefore propose and introduce a systematic nomenclature with guidelines for name selection of new components that will greatly facilitate communication and understanding in this rapidly developing field of research.

The first T7S-associated protein to be identified was the 6-kD early secreted antigenic target ESAT-6 [23]. This small, highly immunogenic protein lacks a classical N-terminal signal sequence and is present in large amounts in the culture filtrate of M. tuberculosis [23], but is missing from the closely related attenuated live vaccine Mycobacterium bovis bacille Calmette-Guérin (BCG) [24] due to the deletion of region of difference 1 (RD1) [25]. ESAT-6 and its protein partner, the 10-kD culture filtrate protein CFP-10 [26], form a 1:1 protein complex [27] that involves hydrophobic interaction [18,28]. Secretion of ESAT-6 and CFP-10 is required for the pathogenicity of M. tuberculosis [29–31]. The absence of ESAT-6 secretion is responsible in part for the attenuation of the BCG and Mycobacterium microti vaccines [13,32,33], as well as for the decrease in virulence of the attenuated M. tuberculosis H37Ra strain [34].

In M. tuberculosis, ESAT-6 and CFP-10 belong to the WXG100 family of 23 small secreted proteins that share a size of approximately 100 amino acids, a helical structure, and a characteristic hairpin bend formed by the conserved Trp-Xaa-Gly (W-X-G) motif [35]. The genes encoding these proteins, many of which represent immunodominant T cell antigens [36], are called esx genes in M. tuberculosis (esx-W, Table 1) and are arranged in tandem pairs at 11 genomic loci [37]. In five of these genomic loci (ESX-1–ESX-5), the esx genes are flanked by genes coding for components of secretion machineries involved in the export of the corresponding ESX proteins (Figure 1). These proteins constitute the major building blocks of the T7S systems [11,12,15,16,19]. Four of these regions are also characterized by the presence of genes encoding PE and/or PPE proteins (Figure 1, Table 2), named after their characteristic N-terminal motifs proline-glutamic acid (PE) and proline-proline-glutamic acid (PPE) [38]. Apart from genes localized in these core ESX regions, additional genes situated elsewhere on the chromosome may be required for the function of T7S systems. For example, the rv3616c-rv3614c genes are required for

Citation: Bitter W, Houben ENG, Bottai D, Brodin P, Brown EJ, et al. (2009) Systematic Genetic Nomenclature for Type VII Secretion Systems. PLoS Pathog 5(10): e1000507. doi:10.1371/journal.ppat.1000507
Editor: Glenn F. Rall, The Fox Chase Cancer Center, United States of America
Published: October 30, 2009
Copyright: © 2009 Bitter et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Funding: This work has received funding from the European Community’s Seventh Framework Programme (FP7/2007–2013) under grant agreement n 201762. In addition, the authors acknowledge their individual external funding for T7S/ESX related research. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Competing Interests: The authors have declared that no competing interests exist.
secretion of ESAT-6 and CFP-10 by ESX-1 [39–41].

Apart from members of the *M. tuberculosis* complex, the ESX-1 cluster is also present in a range of mycobacteria, including *Mycobacterium kansasii* [23] and *Mycobacterium leprae* [42]. However, experimental work has mainly focused on the ESX-1 system of *Mycobacterium marinum* [21,22,43–47], a fish pathogen that shows high homology in its ESX loci with *M. tuberculosis* [48], and the fast grower *Mycobacterium smegmatis* [49–51]. *M. marinum* has also been used to define a role for the paralogue system ESX-5, which is required for the secretion of PE and PPE proteins [16,52,53]. For the remaining ESX-2, ESX-3, and ESX-4 systems, only very limited predictions of their putative functions can be made. ESX-3 transcrip-tome data suggest that this system is involved in iron/zinc homeostasis [54,55], which would be consistent with the essential role of ESX-3 in *M. tuberculosis* [56]. The putative functions of ESX-2 and ESX-4 remain unknown. ESX-4, which harbors a smaller number of genes than other ESX loci (Table 2), appears to represent the most ancestral T7S system in mycobacteria [12]. This hypothesis is based on the observation that ESX-4-like loci are the only ESX clusters that are found in other high GC Gram-positive bacteria, suggesting that the last common ancestor of mycobacteria already harbored an ESX-4 T7S system. Other ESX clusters may have evolved later by gene duplication and gene diversification events. However, the finding that *Nocardia farcinica* [http://nocardia.nih.go.jp/](http://nocardia.nih.go.jp/) contains two T7S systems, one orthologous to ESX-4 and one locus that shows some similarity to all the conserved components of larger T7S systems, suggests that evolution of T7S systems is more complex than previously anticipated. This second

| Gene Family | ESX-1 | ESX-2 | ESX-3 | ESX-4 | ESX-5 |
|-------------|-------|-------|-------|-------|-------|
| ESAT-6      | esxA (esat-6, rv3875) | esxC (rv3890c) | esxH (cfp7, tb10.4, rv2088) | esxT (rv3444c) | esxN (mtb9.9A, Rv1793) |
| CFP-10      | esxB (lhp, cfp-10, rv3874) | esxD (rv3891c) | esxG (tb9.8, rv2087) | esxU (rv3445c) | esxM (tb11.0, rv1792) |
| ESAT-6 homologues elsewhere in the genome | esxR (tb10.3, rv3019c), esxQ (tb5.9, rv3017c) | esxL (mtb9.9D, rv1037c), esxK (mtb9.9C, rv1198), esxO (mtb9.9E, rv2346c), esxV (mtb9.9D, rv3619c) | esxE (rv3904c) |
| CFP-10 homologues elsewhere in the genome | esxS (rv3020c) | esxJ (tb11.0, Rv1038c), esxK (tb11.0, Rv1197), esxF (rv2347c), esxW (rv3620c) |

Table 1. Overview of ex Genes (WXG100 Family) of *M. tuberculosis* H37Rv, Also Showing Previously Used Gene Names in Brackets.

- Only genes that have homologues in at least four of the mycobacterial ESX systems will get a general name, whereas the locus-specific genes have a more restricted name reflecting their specificity. The reason for this distinction is that the conserved genes are most likely to represent the core components of the secretion system. Moreover, all of the conserved ESX-1 components have been shown to be essential for ESAT-6/CFP-10 secretion in at least one of the mycobacterial species studied (See below). In contrast, many of the locus-specific genes encode secreted proteins, as has been shown for the ESX-1 system (see below). Furthermore, in *M. leprae*, an organism with an extremely reductive evolution of its genome, almost all of the non-conserved ESX-1 components are pseudogenes, whereas all of the conserved components seem to be intact [42].

- The three letter acronym for the conserved components will be *ex*, for ex conserved component (Figure 1, Table 2). This abbreviation has not been used for other genes in bacteria.

- The ESAT-6 and CFP-10 encoding genes, *esxA* and *esxB*, respectively, and the other *ex* genes (Table 1) will not be renamed. These gene names are informative, well-accepted, and frequently used in the literature. Furthermore, the *ex* gene products seem to be secreted proteins and do not seem to be components of the secretion system itself, although their presence is required for the secretion of other substrates. The same reasoning is used for the *pe* and *ppe* genes. Four of the five systems harbor *pe* and *ppe* genes, but for the moment their functions within the T7S systems remain uncertain. Furthermore, various mycobacterio...
Figure 1. Genetic organization of the 5 ESX loci and the espA operon in *M. tuberculosis* H37Rv with the proposed nomenclature and predicted cellular localization of the conserved ESX gene products and their interactions. (A) Genetic organization. (B) Model. The abbreviation ecc stands for esx conserved component, whereas esp stands for ESX-1 secretion-associated proteins. The topology of the different proteins in the cytoplasmic membrane shown in (B) refers to the ESX-1 cluster and is based on predictions made using the MEMSAT3 algorithm [60]. Note that the channel drawn in the outer membrane of our model refers to a hypothetical pore, whose existence has not been experimentally demonstrated.

doi:10.1371/journal.ppat.1000507.g001
The alphabetic suffix of conserved genes will be based on the gene order in the paradigm ESX-1 system (see Figure 1). This decision is mainly based on the fact that the ESX-1 system is the most studied. The gene order of the different T7S systems is highly variable and it is therefore difficult to propose a logical ordering that would be satisfactory for all systems. The genes of ESX-2/-3/-4 and ESX-5 will therefore be named according to their parologue in ESX-1 (Table 2 and Table S1), allowing for a direct and relevant comparison. The gene names of each mycobacterial T7S will include a numeral suffix indicating the ESX cluster to which this gene belongs. In order to avoid confusion with numbering of alleles, the ESX cluster number is indicated in subscript. As shown in Figure 1, the first conserved gene of the ESX-1 cluster will be eccA.

- In some of the T7S clusters, the gene encoding the FtsK/SpoIIIE-like protein is split in two genes. Since these gene products clearly form a functional unit, as has also been shown for the two FtsK/SpoIIIE-like proteins of the ESX-1 system [14], the split genes will get a lower case alphabetic suffix, i.e., eccCa1 and eccCt1 for the ESX-1 system of M. tuberculosis (Figure 1 and Table 2).

- When working with several different organisms, it can also be useful to indicate the origin of the respective genes. For this we recommend using a two-letter subscript at the end of the gene name. For example, the orthologues of the M. tuberculosis genes eccCa1 and eccCt1 would be eccCa1mt and eccCt1mt in M. smegmatis.

- The gene names can be converted into their proteins by capitalization, e.g., EccCa1. Alternatively, once the true function of a protein is known, the name could be changed to indicate this function, as has been done for the secretins of type II and type III secretion systems. If in the future new genes are identified that are essential for the functioning of several T7S systems, these genes could be named similarly using the next alphabetical suffix (eccG, eccH, etc.).

- As discussed above, in addition to the conserved genes, there are also region-specific genes. The role of these genes in ESAT-6/CFP-10 secretion is not entirely clear: some of the encoded proteins seem to be involved in the secretion of T7S substrates in M. marinum, whereas their orthologues show less or no effect on secretion in M. tuberculosis. Recently, it has been shown that a subset of these proteins are in fact also substrates of the ESX-1 system. Thus far, four ESX-1 substrates have been identified in addition to ESAT-6 and CFP-10. These substrates are called EspA [39], EspB

### Table 2. New and Old Nomenclature of the Different Esx Conserved Components (ecc Genes) and Genes Encoding ESX-1 Secretion-Associated Proteins (esp Genes) of the T7S Systems of M. tuberculosis H37Rv.

| New Gene Namea | Putative Function of Gene Products | Previously Proposed Gene Names |
|---------------|------------------------------------|-------------------------------|
|               |                                    | ESX-1 | ESX-2 | ESX-3 | ESX-4 | ESX-5 |
| eccA          | AAA+ ATPase                        | rv3868 | rv3884c | rv0282 | -     | rv1798 |
| eccB          | Transmembrane protein (1 TM)       | rv3869 | rv3895c | rv0283 | rv3450c | rv1782 |
| eccC          | FtsK/SpoIIIE-like transmembrane protein (1–3 TMs) | - | rv3894c | rv0284 | rv3447c | - |
| eccCa         | FtsK/SpoIIIE-like transmembrane protein (1–3 TMs) | rv3870 | rv3871snm1 | - | - | - |
| eccCb         | FtsK/SpoIIIE-like transmembrane protein (1–3 TMs) | rv3871 | snm2 | - | - | - |
| eccD          | Transmembrane protein (10–11 TMs)  | rv3877 | snm4 | rv3887c | rv0290 | rv3448c | rv1783 |
| eccE          | Transmembrane protein (2 TMs)      | rv3882c | rv3885c | rv0292 | - | rv1795 |
| mycP          | Subtilisin-like serine protease (mycosin) (1 TM) | rv3883c | rv3886c | rv0291 | rv3449c | rv1796 |
| espA          | Secreted protein                   | rv3616c | - | - | - | - |
| espB          | Secreted protein                   | rv3881c | - | - | - | - |
| espC          | Secreted protein                   | rv3615c | - | - | - | - |
| espD          | Unknown                            | rv3614c | - | - | - | - |
| espE          | Secreted protein                   | rv3864 | - | - | - | - |
| espF          | Secreted protein                   | rv3865 | - | - | - | - |
| espG          | Soluble protein                    | rv3866 | - | rv3889c | rv0289 | - |
| espH          | Unknown                            | rv3867 | - | - | - | - |
| espI          | Pro and Ala rich protein           | rv3876 | snm3 | - | - | - |
| espJ          | Unknown                            | rv3878 | - | - | - | - |
| espK          | Pro and Ala rich protein           | rv3879c | - | - | - | - |
| espL          | Unknown                            | rv3880c | - | - | - | - |
| espR          | Regulation                         | rv3849 | - | - | - | - |

The number of transmembrane domains varies depending on the prediction programme used (for details see Table S2).

The numeral suffix indicating the ESX cluster to which this gene belongs is not shown in this table.

doi:10.1371/journal.ppat.1000507.t002

The gene names can be converted into their proteins by capitalization, e.g., EccCa1. Alternatively, once the true function of a protein is known, the name could be changed to indicate this function, as has been done for the secretins of type II and type III secretion systems. If in the future new genes are identified that are essential for the functioning of several T7S systems, these genes could be named similarly using the next alphabetical suffix (eccG, eccH, etc.).
3. Camacho LR, Ensergueix D, Perez E, Gicquel B, Hoffmann C, Leis A, Niederweis M, Plitzko JM, Trivedi OA, Arora P, Vats A, Ansari MZ, Pugsley AP (1993) The complete general secretion system of Mycobacterium tuberculosis complex lipid determines tissue-specific replication of M. tuberculosis in their native state. J Bacteriol 190: 6428–6438.

4. Abdallah A, Gey van Pittius N, Champion P, Cox J, Lauxinck J, et al. (2007) ESAT-6 from Mycobacterium avium, reveals key residues involved in secretion, complex formation, virulence, and immunogenicity. J Biol Chem 280: 33953–33959.

5. Pugsley AP (1995) The complete general secretory pathway in gram-negative bacteria. Microbiol Rev 57: 50–108.

6. Wick HG, Harboe M (1992) The antigen 85 complex: a major secretion product of Mycobacterium tuberculosis. Microbiol Rev 56: 648–661.

7. Brauning D, Espinosa BJ, Chanh J, Belisle JT, Jacobs WR Jr (2003) SecA2 functions in the secretion of superoxide dismutase A and in the virulence of Mycobacterium tuberculosis. Mol Microbiol 48: 453–464.

8. McDonough JA, McCann JR, Tekippe EM, Silverman JS, Rigel NW, Braunstein M (2008) Identification of functional Tat signal sequences in Mycobacterium tuberculosis proteins. J Bacteriol 190: 6428–6438.

9. Saint-Joanis B, Demangel C, Jackson M, Brodin P, Marsollier L, et al. (2006) Inactivation of Rv2532c, a substrate of the twin arginine translocation (Tat) system of Mycobacterium tuberculosis, increases beta-lactam susceptibility and virulence. J Bacteriol 188: 6669–6679.

10. Gey Van Pittius NC, Gamieldien J, Hide W, Niles M, et al. (2005) Functional analysis of early secreted antigenic target-6, the dominant T-cell antigen of Mycobacterium tuberculosis, reveals key residues involved in secretion, complex formation, virulence, and immunogenicity. J Biol Chem 280: 33953–33959.

11. Champion PA, Stanley SA, Champion MM, Brown EJ, Cox JS (2006) C-terminal signal sequence promotes virulence factor secretion in Mycobacterium tuberculosis. Science 313: 1632–1636.

12. de Jonge MJ, Plehau-Arnautad G, Fretz MM, Romain F, Bottai D, et al. (2007) ESAT-6 from Mycobacterium tuberculosis dissociates from its putative chaperone CFP-10 under acidic conditions and exhibits membrane-lysing activity. J Bacteriol 189: 6028–6034.

13. Smith J, Manoranjan J, Pan M, Bohsali A, Xu J, et al. (2000) Evidence for pore formation in host cell membranes by ESX-1-secreted ESAT-6 and...
its role in *Mycobacterium marinum* escape from the vacuole. Infect Immun 76: 5478–5487.

22. Carlsson F, Joshi SA, Rangell L, Brown EJ (2009) Polar localization of virulence-related Est-1 secretion in mycobacteria. PLoS Pathog 5: e1000283. doi:10.1371/journal.ppat.1000283.

23. Sorensen AL, Nagai S, Houen G, Andersen P, Andersen AB (1995) Purification and characterization of a low-molecular-mass T-cell antigen secreted by *Mycobacterium tuberculosis*. Infect Immum 63: 1710–1717.

24. Harboe M, Oettinger T, Wiker HG, Rosenkranz I, Andersen P (1996) Evidence for occurrence of the ESAT-6 protein in *Mycobacterium tuberculosis* and virulent *Mycobacterium bovis* and for its absence in *Mycobacterium bovis* BCG. Infect Immun 64: 16–22.

25. Hsu T, Hingley-Wilson SM, Chen B, Chen M, Mathur SK, Hickey MJ, Majlessi L, Marsollier I, de Jonge MJ, Brodin P, Brosch R, Huerre M, Cole ST (2002) ESAT-6 secretion and specific T cell recognition by PhoP. PLoS Pathog 4: e30. doi:10.1371/journal.ppat.0040333.

26. Skiot RL, Oettinger T, Rosenkranz I, Renshaw PS, Lightbody KL, Veverka V, et al. (2006) Evolution and expansion of the *Mycobacterium tuberculosis* PE and PPE multigene families and their association with the duplication of the ESAT-6 (esx) gene cluster regions. BMC Evol Biol 6: 93.

27. Renshaw PS, Panagiotidou P, Whelan A, Hickey MJ, Singh DC, Mahairas GG, Sabo PJ, Hickey MJ, Singh DC, et al. (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393: 537–544.

28. Seyit FB, Policastro W, Stevens L, Ehlers MR, et al. (2000) The primary mechanism of secretion of proteins required for mycobacterial virulence. Proc Natl Acad Sci U S A 102: 10676–10681.

29. MacGurn JA, Raghavan S, Stanley SA, Cox JS (2005) A non-RD1 gene cluster is required for *M. tuberculosis* virulence. Mol Microbiol 57: 1659–1672.

30. Raghavan S, Manzanillo P, Chan K, Dowe, Cox JS (2008) Secreted transcription factor controls *Mycobacterium tuberculosis* virulence. Nature 454: 717–721.

31. Raghavan S, Manzanillo P, Chan K, Dowe, Cox JS (2008) Secreted transcription factor controls *Mycobacterium tuberculosis* virulence. Nature 454: 717–721.

32. Flodmark C, Oppedal K, Hindsen H, Halse T, Hysing I, Vondrus F, Veszpremy Z, et al. (2006) ESX-1 protein co-secretes with CFP-10/ESAT-6 and is necessary for inhibiting phagosome maturation. Cell Microbiol 8: 1417–1429.

33. Muskett FW, Kelly G, et al. (2005) Structure and function of the complex formed by the tuberculosis virulence factor *Mycobacterium tuberculosis* ESAT-6 and a novel low-molecular-mass culture filtrate protein (CFP-10). Microbiology 141: 3195–3203.

34. Bottai D, et al. (2006) Dissection of ESAT-6, CFP-10, and the ESAT-6*CFP-10* operon encoding ESAT-6 and a novel low-molecular-mass culture filtrate protein (CFP-10) contributing to the attenuation of *M. tuberculosis* infection. Proc Natl Acad Sci U S A 102: 10640–10645.

35. Gey van Pittius NC, Sampson SL, Lee H, Kim Y, et al. (2006) The role of the RD54 region superfamily in ESAT-6 secretion and specific T cell recognition by PhoP. PLoS Pathog 4: e30. doi:10.1371/journal.ppat.0040333.

36. Flodmark C, Oppedal K, Hindsen H, Halse T, Hysing I, Vondrus F, Veszpremy Z, et al. (2006) ESX-1 protein co-secretes with CFP-10/ESAT-6 and is necessary for inhibiting phagosome maturation. Cell Microbiol 8: 1417–1429.

37. Cole ST, Eiglmeier K, Parkhill J, James KD, et al. (2000) A non-RD1 gene cluster is required for *Mycobacterium tuberculosis* virulence. Proc Natl Acad Sci U S A 102: 10676–10681.

38. Gey van Pittius NC, Sampson SL, Lee H, Kim Y, et al. (2006) The role of the RD54 region superfamily in ESAT-6 secretion and specific T cell recognition by PhoP. PLoS Pathog 4: e30. doi:10.1371/journal.ppat.0040333.

39. Davis JM, Ramakrishnan L (2009) The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell 136: 37–49.

40. Stinear TP, Seemann T, Harrison PF, Jenkin GA, Davies JK, et al. (2008) Insights from the complete genome sequence of *Mycobacterium marinum* on the evolution of *Mycobacterium tuberculosis*. Genome Res 18: 729–741.

41. Raghavan S, Manzanillo P, Chan K, Dowe, Cox JS (2008) Secreted transcription factor controls *Mycobacterium tuberculosis* virulence. Mol Microbiol 57: 1659–1672.

42. Cole ST, Eiglmeier K, Parkhill J, James KD, et al. (2000) A non-RD1 gene cluster is required for *Mycobacterium tuberculosis* virulence. Proc Natl Acad Sci U S A 102: 10676–10681.