EMBO reports

correspondence

One bacterial effector with two distinct catalytic activities by different strains

The causative agent of Legionnaires' disease, *Legionella pneumophila*, is a master manipulator of various eukaryotic hosts ranging from unicellular amoebae to mammals [1]. This intracellular pathogen, which invades macrophages in mammalian hosts, evades the default endosome–lysosome pathway and remodels the phagosome, enclosing it in an endoplasmic reticulum-derived *Legionella*-containing vacuole (LCV). *L. pneumophila* facilitates this by hijacking a myriad of eukaryotic cellular functions through translocation of around 300 effectors into the host cell by the Dot/Icm type IVB secretion apparatus. More than 70 of the injected effector proteins contain eukaryotic-like domains, including the ankyrin repeat, Sel1, F-box, SET, U-box and leucine-rich repeats, which suggests *L. pneumophila* hijacks and manipulates various eukaryotic processes through molecular mimicry.

Elegant work by Rolando *et al* [2] and Li *et al* [3] described a new effector, designated RomA or LegAS4, respectively, which exhibits molecular mimicry of host SET-domain-containing histone methyltransferases to hijack host transcriptional programming by methylating histones on specific lysine residues [2,3]. Histone methylation has a crucial role in epigenetic control of gene transcription. Prior to these findings, no intracellular pathogen had been shown to directly affect chromatin architecture to alter the host cell transcriptional landscape. Rolando and Li both show that RomA/LegAS4 is a histone methyltransferase, but that the functional phenotype of RomA/LegAS4 is different in the two strains of *L. pneumophila* used in the two studies. Rolando *et al* primarily used the Paris strain of *L. pneumophila* and show that RomA localizes to the host nucleus in which it catalyses a new histone methylation mark, H3K14, which replaces H3K14 acetylation. This new methylation mark has not been previously shown to occur in mammalian cells and results in global gene transcriptional repression, particularly in genes that are involved in innate immunity. Furthermore, deletion of RomA renders the Paris strain defective in intracellular growth within both macrophages and amoebae, suggesting that repression of host global transcription is important for *L. pneumophila* pathogenesis. By contrast, Li *et al* used the *L. pneumophila* Philadelphia-derivative Lp02 strain to characterize LegAS4. However, unlike RomA from the Paris strain, LegAS4 localizes predominately to the nucleolus in which it catalyses H3K4 methylation to promote increased transcription of rDNA genes through direct interaction with heterochromatin binding protein 1, and does not have a global effect on host gene transcription. Interestingly, Ronaldo *et al* did not find any evidence for the RomA-mediated H3K4 methylation seen with LegAS4, but did observe an increase in H3K14 methylation in cells infected with the Lp02 strain. This suggests that LegAS4 might catalyse this new histone methylation mark, but that further analyses are needed.

How can a highly homologous effector have such discrepant phenotypes in two different strains of *L. pneumophila*? An amino acid sequence alignment of RomA and LegAS4 reveals two major differences (Fig 1). First, RomA is missing the first 13 amino-terminal amino acids present in LegAS4. Second, an eight amino acid stretch spanning positions 66–74 and 80–87 in RomA and LegAS4, respectively, shows weak sequence homology with only a lysine residue shared in this region (Fig 1). It is possible these two regions of difference, which occur upstream from the conserved catalytic SET domain, might alter the structure of RomA from LegAS4 resulting in a change in histone methyltransferase substrate specificity, thus promoting H3K14 methylation compared with H3K4 methylation. Another factor to consider is the difference in nuclear localization of RomA and LegAS4. Although both RomA and LegAS4 localize to the nucleus, LegAS4 has an intense localization to the nucleolus, which is not seen for RomA. The ability of LegAS4 to localize to the nucleolus is dependent on its tandem nuclear localization signal (NLS) at amino acid positions 21–60, which alone is sufficient to target GFP to the nucleolus. RomA has three individual amino acid differences from LegAS4 in its corresponding NLS (Fig 1). Nuclear and nucleolar localization signals are similar and characterized by stretches of basic amino acids such as lysine [4]. It is possible that the three amino acid differences in the NLS of LegAS4 are sufficient to target this effector to the nucleolus, in which it can act directly on rDNA gene transcription, rather than have an effect on global gene transcription as with RomA, which does not target the nucleolus. Taken together,

```
LegAS4  MIILYDFLQESSVMPRSKNDSKLKKKSALQSKFKEQQLNHGSKEHKSKFKFSQRKAKKKG 60
RomA    -------------MPRSKNDSNLKKKSALQSKFKEQQWNHGSKEHKSKFKFTQRKAKKKG 47
                     ********:*************** ************:********

LegAS4  PGMTHLPGNIYTLFTPVNGPKSKDEWIDTSKIMLDLHIDNMSSSDYIPSAIDRTDLVMVQ 120
RomA    PGMTHLPGNIYTLFTPVNGLKNNAQLTDTSKIMVNLHIDNMSSSDYIPSAIDRTDLVIIQ 107
        ****************** *.: :  ******::**********************:**
```

Fig 1 | Clustal omega alignment of the amino-terminal region of LegAS4 and RomA in *Legionella pneumophila* Lp02 and Paris, respectively. Highlighted in yellow are the 13 N-terminal amino acids found in LegAS4 but missing in RomA. Highlighted in green is the NLS region, which shows three amino acid differences between LegAS4 and RomA, and highlighted in blue is an eight amino acid stretch that shows poor sequence homology. NLS, nuclear localization signal.

structural differences between LegAS4 and RomA probably explain the differential nucleolar protein targeting and distinct functional phenotypes of homologous effectors. It is also important to note that mass spectrometric analyses were used by Rolando *et al*, which identified the previously unknown H3K14 methylation mark, and this could have been missed in the Li *et al* study because they did not use this technique.

L. pneumophila encounters a diverse range of potential amoebal hosts, and consequently each strain harbours a distinct cadre of effectors that reflects adaptation to distinct primitive eukaryotic hosts in the aquatic environment. Of the approximately 300 known effectors of *L. pneumophila,* roughly 100 are shared between different strains [5], but so far differences between the amino acid sequences of the shared effectors have not been well characterized. It is clear from RomA and LegAS4 that minor differences in amino acid sequence might have significant consequences for both cellular localization and function in the host cell. For an example, the F-box AnkB effector of strain 130b/AA100 of *L. pneumophila* is anchored to the LCV membrane through a carboxy-terminal 'CaaX' farnesylation motif, in which it mediates decoration of the LCV with Lys 48-linked polyubiquitinated proteins that are degraded by the proteasomes, generating a surplus of host amino acids needed for bacterial proliferation [6,7]. Interference with farnesylation of AnkB of the 130b/AA100 strain renders the effector non-functional and the bacteria become attenuated *ex vivo* and *in vivo* [6]. By contrast, the C-terminus of the AnkB homologue in the Paris strain is truncated for the last 18 amino acids, including the farnesylation motif, and the five C-terminal amino acids of the Paris AnkB are different from the 130b/AA100 [8]. This has probably resulted in the lack of anchoring of AnkB of the Paris strain to the LCV membrane. This structural difference between the two AnkB homologues probably explains the differences in cellular localization and function between the homologous effectors in the two strains. It is becoming clear that homologous effectors in different strains of *L. pneumophila* might have evolved to have distinct roles to hijack cellular processes, and it is possible that a similar phenomenon also occurs in other species of bacterial pathogens. Therefore, it is becoming increasingly important to analyse how homologous bacterial effectors in different pathogenic strains each affect the host, as this will provide insight into how pathogens hijack host processes and eukaryotic cell biology in general.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Al-Quadan T, Price CT, Abu Kwaik Y (2012) *Trends Microbiol* **20:** 299–306
2. Rolando M *et al* (2013) *Cell Host Microbe* **13:** 395–405
3. Li T *et al* (2013) *EMBO Rep* [Epub ahead of print] doi:10.1038/embor.2013.86
4. Scott MS *et al* (2010) *Nucleic Acids Res* **38:** 7388–7399
5. Schroeder GN *et al* (2010) *J Bacteriol* **192:** 6001–6016
6. Price CT *et al* (2010) *J Exp Med* **207:** 1713–1726
7. Price CT *et al* (2011) *Science* **334:** 1553–1557
8. Lomma M *et al* (2010) *Cell Microbiol* **12:** 1272–1291

Christopher T.D. Price and Yousef Abu Kwaik are at the Department of Microbiology & Immunology, College of Medicine, University of Louisville, Kentucky, USA. E-mails: ctpric01@louisville.edu; abukwaik@louisville.edu

EMBO *reports* (2013) **14,** 753–754; published online 16 August 2013; doi:10.1038/embor.2013.126

