Did the loss of two-component systems initiate pseudogene accumulation in *Mycobacterium leprae*?

The predilection of the leprosy bacillus for peripheral nerves and cutaneous tissues is indicative of its restricted ability to adapt to microenvironments prevailing in other anatomical sites in the body. No pathogen, *Mycobacterium leprae* included, is likely to self-limit its options in terms of the tissues it targets and proliferates in. Rather, it seems some compelling circumstances would have contributed to its narrow preferences in lifestyle. In a recent report, Madan Babu (2003) suggested that the loss of sigma factors could have initiated the accumulation of pseudogenes in the *M. leprae* genome. It is a very interesting analysis and suggests how normal genes could turn into pseudogenes through non-random events triggered by the loss of sigma factors.

RNA polymerases utilize alternative sigma factors to enable bacteria to express discrete gene sets in response to various physiological and environmental stimuli. *Mycobacterium tuberculosis* has several sigma factors to choose from; its genome encodes 13 (Cole *et al.*, 1998). The importance of sigma factors is highlighted by studies of SigE, SigH and SigF, whose loss has rendered tubercle bacilli sensitive to various environmental stresses (such as heat shock, oxidative stress and growth in macrophages) and in some cases caused an attenuated virulence phenotype (Manganelli *et al.*, 2001, 2002; Raman *et al.*, 2001; Chen *et al.*, 2000; Kaushal *et al.*, 2002). It is noteworthy that *M. leprae* retains only four sigma factor genes (including *sigA*, the primary mycobacterial sigma factor) while losing most of them, including *sigF*, *sigH*, etc. (Cole *et al.*, 2001). *sigF* expression is induced under a variety of conditions, including anaerobiosis in *M. tuberculosis* (Michele *et al.*, 1999; Sherman *et al.*, 2001), and it is tempting to think that the deletion of this and/other sigma factors in *M. leprae* prevents its adaptation to these conditions. Furthermore, σ^F activity can be post-translationally modulated by anti- and anti-anti-sigma factors (Beaucher *et al.*, 2002) in *M. tuberculosis* but not in *M. leprae*, because the latter lost the relevant genes. As a general trend, most ECF sigma factors are co-transcribed with one or more negative regulators that function as anti-sigma factors, bind to the sigma factors and inhibit their activity. In response to an environmental stimulus, the sigma factor is released and in association with the core RNA polymerase stimulates transcription. These anti-sigma/sigma pairs are analogous to the histidine sensor kinase/response regulator pairs that comprise typical two-component systems, in that both systems orchestrate an appropriate cellular response to environmental signals. Furthermore, there are many examples of signal transduction systems regulating the activity of sigma factors in response to environmental stresses (Paget *et al.*, 1999; Klauck *et al.*, 2001; Akbar *et al.*, 2001; Nizan-Koren *et al.*, 2003).

So is the loss of sigma factors all there is to the accumulation of pseudogenes in *M. leprae*? We propose that the loss of specific global signal transduction systems such as eukaryotic-like Ser/Thr protein kinases or two-component systems could have severely compromised *M. leprae*'s capacity to adapt to environments that individual systems were designed to sense and respond to. The loss in this ability would over time result in the decay of genes, including sigma factor(s), participating in that specific response. To illustrate this point, let us consider the hypoxia response of mycobacteria. Both the slow- and rapid-growing species, including *M. tuberculosis*, *Mycobacterium bovis* and *Mycobacterium smegmatis*, adapt to conditions of oxygen limitation (Wayne & Sohaskey, 2001) by a molecular mechanism that is conserved in them

Table 1. *M. leprae* orthologues to hypoxia-responsive genes of *M. tuberculosis*

Genes that displayed ⩾3·0-fold change in signal from baseline during microarray hybridization analysis (Sherman *et al.*, 2001) were taken for this analysis.

Hypoxia-responsive gene	Gene annotation in *M. tuberculosis**	Hypoxia response	*M. leprae* orthologue†
Induced genes			
Rv0079	HP	+9·2	−
Rv0080	HP	+8·2	−
Rv0081	Transcription regulator	+3·8	−
Rv0350	DnaK, heat-shock chaperone protein	+3·6	+
Rv0440	60 kDa GroEL2 Chaperonin2	+3·0	+
Rv0569	CHP	+16·2	−
Rv0570	NrdZ, ribonucleotide reductase	+3·0	+
Rv0572c	HP	+8·0	−
Rv1592c	CHP	+3·4	−
Rv1733c	Possible membrane protein	+15·5	−
Rv1734c	HP	+5·1	−
Rv1736c	NarX, fused nitrate reductase	+3·3	−
Rv1737c	NarK2, nitrite extrusion protein	+7·1	+
Rv1738	CHP	+50·4	−
Rv1739c	Possible sulfate transporter	+4·1	+
Rv1813c	CHP	+12·7	−
Rv1996	CHP (Usp)	+13·7	−
Rv1997	CtpF, probable cation transport ATPase	+7·5	+
Rv1998c	CHP	+8·6	−
Rv2003c	CHP	+10·3	−
Rv2005c	CHP (Usp)	+9·2	−
Rv2006	OtsB, trehalose-6-phosphate phosphatase	+4·0	+
Rv2007c	FdxA, ferredoxin	+24·1	+
Rv2028c	CHP (Usp)	+3·5	−
Rv2029c	PfkB, phosphofructokinase II	+12·2	−
Rv2030c	HP	+10·6	−
Rv2031c	Acr (HspX)	+14·6	+
Rv2032	CHP	+45·2	−
Rv2428	AhpC, alkyl hydroperoxide reductase	+4·2	+
Rv2623	CHP (Usp)	+7·3	−
Rv2624c	CHP (Usp)	+19·7	−
Rv2625c	CHP	+6·9	−
Rv2626c	CHP	+40·6	−
Rv2627c	CHP	+11·9	−
Rv2628	HP	+5·2	−
Rv2629	HP	+7·4	−
Rv2630	HP	+4·2	−
Rv2659c	PhiRV2 integrase	+4·1	−
Rv2780	L-Alanine dehydrogenase	+9·8	+
Rv3126c	HP	+22·7	−
Rv3127	CHP	+36·0	−
Rv3128c	CHP	+15·2	−
Rv3129	CHP	+21·0	−
Rv3130c	HP	+11·9	−
Rv3131	CHP	+4·6	−
Rv3132c	DevS, histidine kinase	+9·8	−
Rv3133c	DevR/DosR, response regulator	+11·9	−
Rv3134c	CHP (Usp)	+11·5	−
Rv3269	Heat-shock protein	+3·7	+
Rv3615c	CHP	+3·7	+

Table 1. cont.

Hypoxia-responsive gene	Gene annotation in *M. tuberculosis**	Hypoxia response	*M. leprae* orthologue†
Rv3841	BfrB, bacterioferritin	+8·9	+
Rv3842c	GlpQ, glycerophosphoryl diester phosphodiesterase	+4·9	+
Rv3853	MenG	+3·1	+
Rv3854c	Probable monooxygenase	+7·0	+
Rv3908	CHP	+4·0	+
Repressed genes			
Rv0694	L-Lactate dehydrogenase	−3·1	+
Rv0710	Small ribosomal subunit protein	−3·0	+
Rv1129c	Transcription regulator	−5·1	−
Rv1130	CHP	−4·6	−
Rv2947c	Pks15, polyketide synthase	−3·1	+
Rv2948c	FadD22, acyl-CoA synthase	−3·8	+
Rv3152	NuoH, NADH dehydrogenase H	−3·4	−
Rv3249c	Transcription regulator	−3·0	−
Rv3250c	Rubredoxin B	−4·4	−
Rv3251c	Rubredoxin A	−4·5	−
Rv3252c	Possible alkane-1-monooxygenase	−4·9	−
Rv3727	Similar to phytoene dehydrogenase precursor	−3·3	−
Rv3920c	CHP	−3·6	+

*According to Cole *et al.* (1998). Proteins containing Usp domains detected using the INTERPRO SCAN tool are indicated. CHP, conserved hypothetical protein; HP, hypothetical protein.

†An *M. leprae* gene is considered as an orthologue (+) if it is annotated or shows ⩾50 % homology over 90 % of its translated sequence with the *M. tuberculosis* homologue; a minus (−) indicates the gene is a pseudogene or has no significant homology.

and involves a two-component system, DevR–DevS (Sherman *et al.*, 2001; Mayuri *et al.*, 2002; Boon & Dick, 2002; O'Toole *et al.*, 2003), which was first identified in *M. tuberculosis* (Dasgupta *et al.*, 2000). DevR/DosR has recently been shown to regulate the hypoxia-responsive expression of several mycobacterial genes (Boon & Dick, 2002; O'Toole *et al.*, 2003; Park *et al.*, 2003). As mentioned, large-scale changes in gene expression were noted in *M. tuberculosis* cultures exposed briefly to hypoxia. Using the dataset that was generated by microarray RNA expression analysis (Sherman *et al.*, 2001), we performed a simple search in the annotated *M. leprae* genome for orthologues to *M. tuberculosis* genes whose expression was substantially altered under hypoxic conditions (Table 1). The analysis revealed some interesting points. Among the top 68 protein-coding genes analysed, 46 were either pseudogenes or absent from *M. leprae* (Cole *et al.*, 2001) and included those encoding the DevR–DevS two-component system. This translates to a loss of ~68 % of the genes analysed and equals in magnitude to a 69 % loss of sigma factor genes. Furthermore, genes conserved in *M. leprae* included those encoding proteins that are likely to have functions in addition to hypoxic adaptation and included heat-shock and stress-induced proteins, transporters, proteins involved in fatty acid and polyketide biosynthesis and conserved proteins of unknown function. This pattern of loss and conservation is indicative of a selective loss of those genes that are regulated during hypoxia. On this basis, we propose that the primary loss of DevR–DevS triggered the accumulation of mutations in the genes of the hypoxia regulon in *M. leprae*. Apart from DevR–DevS, *M. leprae* has also lost six of 11 orthologues each of *M. tuberculosis* two-component systems, orphan two-component systems and Ser/Thr protein kinases (Cole *et al.*, 2001), the decay of which could have triggered the accumulation of pseudogenes in their respective regulons.

Regardless of whether the deletion of two-component systems preceded that of sigma factor genes or vice versa, the question still remains as to why so many systems regulating adaptive processes were susceptible to gene decay in *M. leprae*. Perhaps by setting in motion the process of decay of regulatory genes and as a consequence of natural selection wherein the retention of useful traits was the key, the emergence of pseudogenes among structural genes under the control of various mutated environmentally responsive regulatory genes was inevitable over time. As a corollary, only those genes were retained that participated in processes absolutely essential to the leprosy bacillus.

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