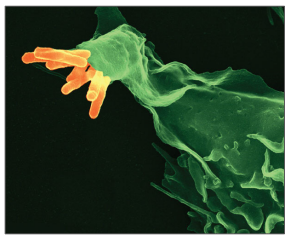
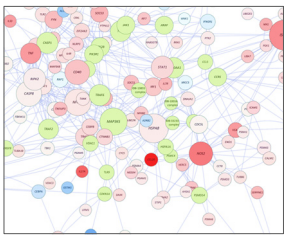
Multi-omics of the bovine host response to *Mycobacterium bovis* infection and tuberculosis disease

Bovine Tuberculosis Host Pathogen Interface Meeting
Stormont Hotel, Belfast, 4th–5th December 2023

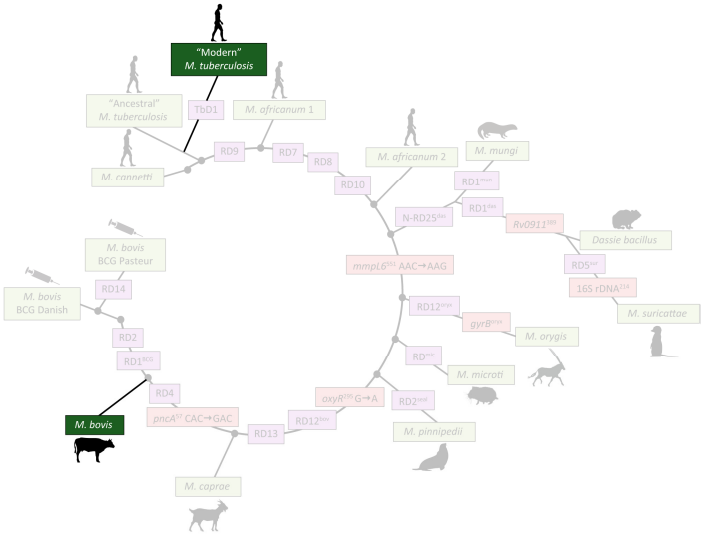
David MacHugh, PhD, Professor of Functional Genomics,
Animal Genomics Group, University College Dublin

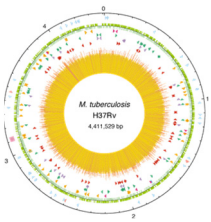
W: people.ucd.ie/david.machugh E: david.machugh@ucd.ie @david_machugh

Steve Gordon (Co-PI)

1

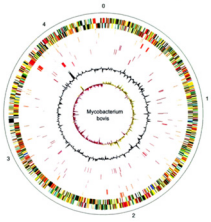
The *Mycobacterium tuberculosis* complex (MTBC)





M. tuberculosis H37Rv
4.41 Mb genome
3,995 genes

2,347 SNPs
~ 0.05% sequence divergence



M. bovis 2122
4.34 Mb genome
3,951 genes

Malone K.M. & Gordon S.V. (2017) *Adv. Exp. Med. Biol.* **1019**, 135-54. DOI: 10.1007/978-3-319-64371-7_7
Faherty O'Donnell S.L., *et al.* (2019) In: *MicroRNAs in Diseases and Disorders*, pp. 105-32. RCS, Cambridge, UK. DOI: 10.1039/9781788016421-00105

2

Maintenance hosts/wildlife reservoirs of *M. bovis* and examples of spillover hosts

Maintenance hosts/wildlife reservoirs

Cattle
(*Bos taurus*)

European badger
(*Meles meles*)

Multiple deer species

Wild boar (*Sus scrofa*)

Brushtail possum
(*Trichosurus vulpecula*)

Examples of spillover hosts

Human
(*Homo sapiens*)

Domestic cat
(*Felis catus*)

Sheep (*Ovis aries*)
and goat (*Capra hircus*)

African buffalo
(*Syncerus caffer*)

White rhino
(*Ceratotherium simum*)

African lion
(*Panthera leo*)

Llama
(*Llama glama*)

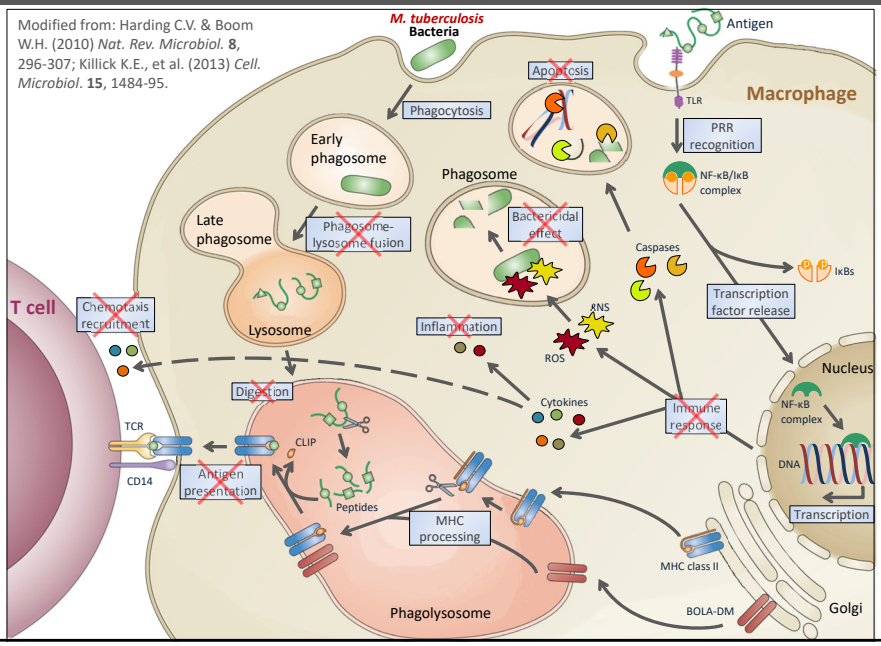
3

The maintenance host for *M. tuberculosis*

Homo sapiens

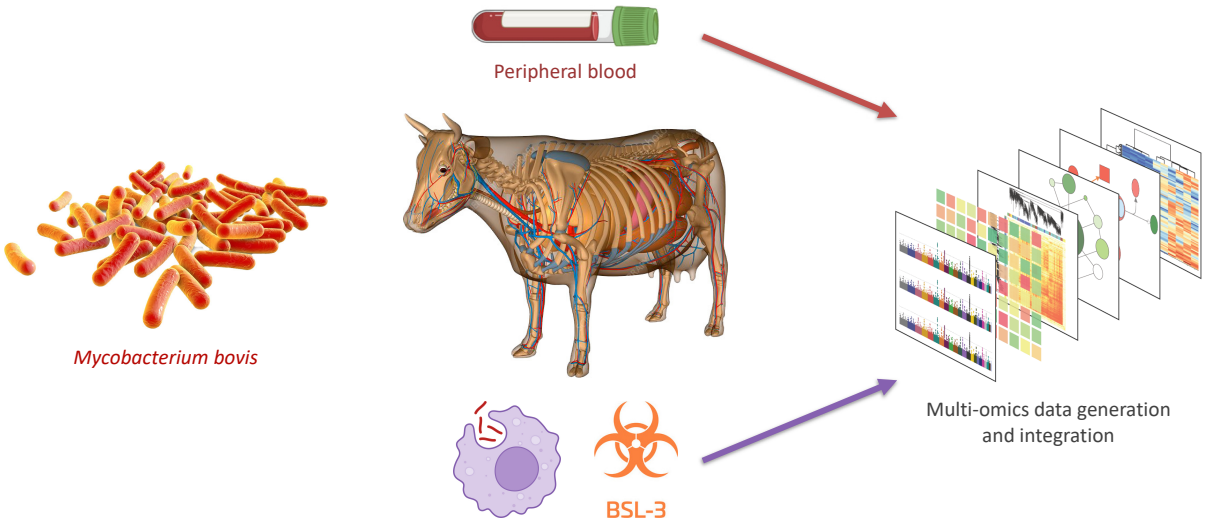
4

M. tuberculosis (and *M. bovis*) and the host alveolar macrophage



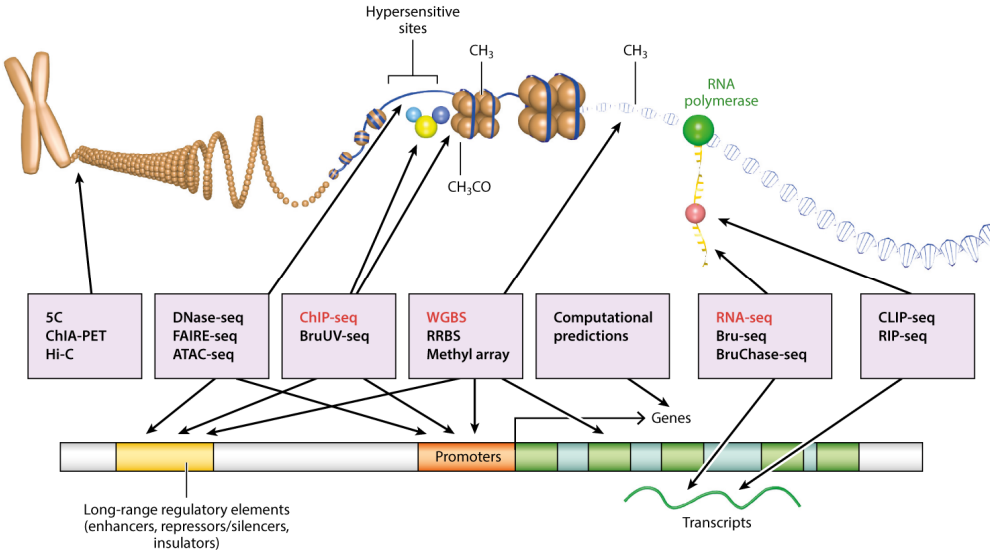
5

Studying host-pathogen interactions for tuberculosis-causing mycobacteria using multi-omics analyses of peripheral blood and alveolar macrophages



6

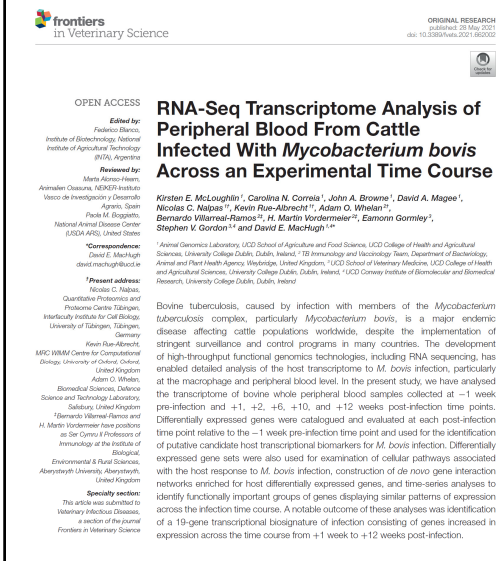
Functional genomics methodologies and technologies (primarily based on high-throughput sequencing - HTS)



Giuffra *et al.* (2019) *Annu. Rev. Anim. Biosci.* **7**, 65-88. DOI: 10.1146/annurev-animal-020518-114913

7

RNA-seq transcriptome analysis of peripheral blood from cattle infected with *Mycobacterium bovis* across an experimental time course



frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 28 May 2021
doi: 10.3389/fvets.2021.662002

OPEN ACCESS

Edited by:
Federico Blanco,
Institute of Biotechnology, National Institute of Agriculture Technology (INTA), Argentina

Reviewed by:
Marta Alonso-Hearn,
Animalien Osasuna, NEIKER-Instituto Vasco de Investigación y Desarrollo Agrario, Spain
Paola M. Boggiatto,
National Animal Disease Center (USDA ARS), United States

***Correspondence:**
David E. MacHugh
david.machugh@ucd.ie

†Present address:
Nicolas C. Nalpas,
Quantitative Proteomics and Proteome Centre Tübingen, Interfaculty Institute for Cell Biology, University of Tübingen, Tübingen, Germany
Kevin Rue-Albrecht,
MRC WIMM Centre for Computational Biology, University of Oxford, Oxford, United Kingdom
Adam O. Whelan,
Biomedical Sciences, Defence Science and Technology Laboratory, Salisbury, United Kingdom
‡Bernardo Villarreal-Ramos and H. Martin Vordermeier have positions as Ser Cymru II Professors of Immunology at the Institute of Biological, Environmental & Rural Sciences, Aberystwyth University, Aberystwyth, United Kingdom

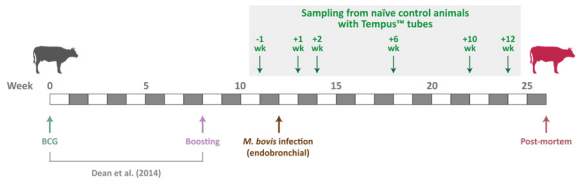
Specialty section:
This article was submitted to Veterinary Infectious Diseases, a section of the journal Frontiers in Veterinary Science

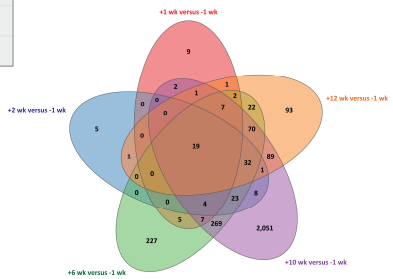
RNA-Seq Transcriptome Analysis of Peripheral Blood From Cattle Infected With *Mycobacterium bovis* Across an Experimental Time Course

Kirsten E. McLoughlin[1], Carolina N. Correia[1], John A. Browne[1], David A. Magee[1], Nicolas C. Nalpas[1†], Kevin Rue-Albrecht[1†], Adam O. Whelan[2†], Bernardo Villarreal-Ramos[3‡], H. Martin Vordermeier[3‡], Eamonn Gormley[3], Stephen V. Gordon[3,4] and David E. MacHugh[1,4]*

[1] Animal Genomics Laboratory, UCD School of Agriculture and Food Science, UCD College of Health and Agricultural Sciences, University College Dublin, Dublin, Ireland, [2] TB Immunology and Vaccinology Team, Department of Bacteriology, Animal and Plant Health Agency, Weybridge, United Kingdom, [3] UCD School of Veterinary Medicine, UCD College of Health and Agricultural Sciences, University College Dublin, Dublin, Ireland, [4] UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

Bovine tuberculosis, caused by infection with members of the *Mycobacterium tuberculosis* complex, particularly *Mycobacterium bovis*, is a major endemic disease affecting cattle populations worldwide, despite the implementation of stringent surveillance and control programs in many countries. The development of high-throughput functional genomics technologies, including RNA sequencing, has enabled detailed analysis of the host transcriptome to *M. bovis* infection, particularly at the macrophage and peripheral blood level. In the present study, we have analysed the transcriptome of bovine whole peripheral blood samples collected at −1 week pre-infection and +1, +2, +6, +10, and +12 weeks post-infection time points. Differentially expressed genes were catalogued and evaluated at each post-infection time point relative to the −1 week pre-infection time point and used for the identification of putative candidate host transcriptional biomarkers for *M. bovis* infection. Differentially expressed gene sets were also used for examination of cellular pathways associated with the host response to *M. bovis* infection, construction of *de novo* gene interaction networks enriched for host differentially expressed genes, and time-series analyses to identify functionally important groups of genes displaying similar patterns of expression across the infection time course. A notable outcome of these analyses was identification of a 19-gene transcriptional biosignature of infection consisting of genes increased in expression across the time course from +1 week to +12 weeks post-infection.





McLoughlin K.E., *et al.* (2021) *Front. Vet. Sci.* **8**, 662002, doi: 10.3389/fvets.2021.662002.

8

RNA-seq transcriptome analysis of peripheral blood from cattle infected with *Mycobacterium bovis* across an experimental time course

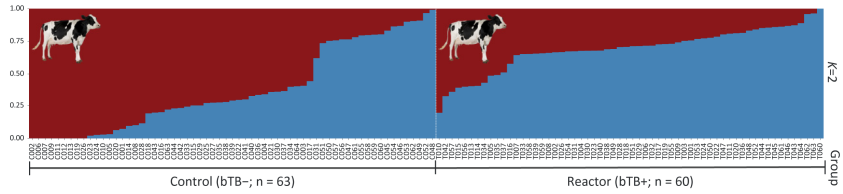
			Male animals					Female animals	
			Animal infection time course - RNA-seq (versus -1 wk)					PBL study	PBL study
			+1 wk	+2 wk	+6 wk	+10 wk	+12 wk	RNA-seq	Microarray
ENSBTAG00000000306	***ITK***	IL2-inducible T-cell kinase	1.28	1.36	1.23	1.37	1.35	1.68	
ENSBTAG00000000507	***NR4A1***	Nuclear receptor subfamily 4, group A, member 1	2.51	3.52	4.49	11.04	2.51	4.04	1.56
ENSBTAG00000001060	***CXCR4***	Chemokine (C-X-C motif) receptor 4	1.99	2.05	2.13	3.09	2.30	3.23	2.48
ENSBTAG00000002758	***THBD***	Thrombomodulin	1.89	2.01	2.76	3.10	1.86	1.50	1.29
ENSBTAG00000003553	***ZFP36L2***	ZFP36 ring finger protein-like 2	1.52	1.58	1.69	1.87	1.52	4.06	
ENSBTAG00000003650	***NR4A2***	Nuclear receptor subfamily 4, group A, member 2	2.49	3.49	4.32	9.96	2.59	7.32	3.22
ENSBTAG00000004305	***RGS16***	Regulator of G-protein signaling 16	2.42	3.09	2.84	4.84	2.27	2.84	1.55
ENSBTAG00000006806	***KRT17***	Keratin 17	5.57	7.94	10.26	14.58	4.16	40.23	5.39
ENSBTAG00000008182	***FOSB***	FBJ murine osteosarcoma viral oncogene homolog B	2.50	4.02	5.05	6.27	2.93		
ENSBTAG00000008353	***CDKN1A***	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.71	2.24	2.59	3.73	2.03	3.01	2.14
ENSBTAG00000009354	***EVI2A***	Ecotropic viral integration site 2A	1.34	1.26	1.43	1.57	1.37		
ENSBTAG00000013125	***PLAUR***	Plasminogen activator, urokinase receptor	3.52	5.30	5.96	13.03	3.81	6.91	4.52
ENSBTAG00000016163	***OSM***	Oncostatin M	2.44	4.03	3.83	6.79	2.61	4.37	
ENSBTAG00000019716	***CXCL8***	chemokine (C-X-C motif) ligand 8	3.09	6.56	5.93	14.19	4.87	5.41	1.70
ENSBTAG00000021766	***HBEGF***	Heparin-binding EGF-like growth factor	3.94	4.18	5.45	9.77	3.00	10.02	
ENSBTAG00000031707	***FRMD6***	FERM domain containing 6	1.29	1.28	1.36	1.41	1.44	2.48	1.30
ENSBTAG00000035224	---	Uncharacterized protein	6.24	5.88	7.06	22.21	6.64		
ENSBTAG00000037608	---	Uncharacterized protein	2.94	3.43	4.08	9.59	3.07	7.83	
ENSBTAG00000039037	***SERPINB4***	Serpin peptidase inhibitor, clade B	3.50	4.90	5.58	12.68	4.24	10.13	

Lowest expression increase — Highest expression increase

McLoughlin K.E., *et al.* (2021) *Front. Vet. Sci.* **8**, 662002, doi: 10.3389/fvets.2021.662002.

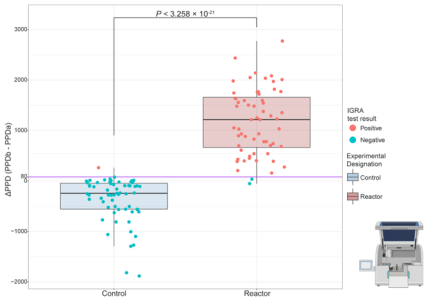
9

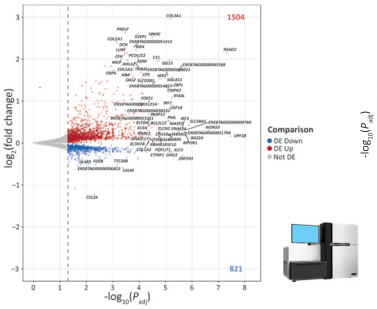
Peripheral blood transcriptomics and systems genetics of cattle infected with *Mycobacterium bovis* sheds light on the immunobiology of tuberculosis disease.

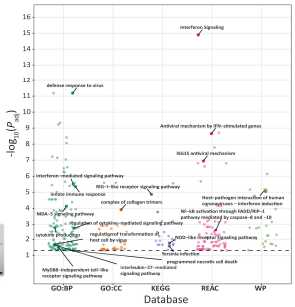


John O'Grady

SFI Centre for Research Training in GENOMICS DATA SCIENCE





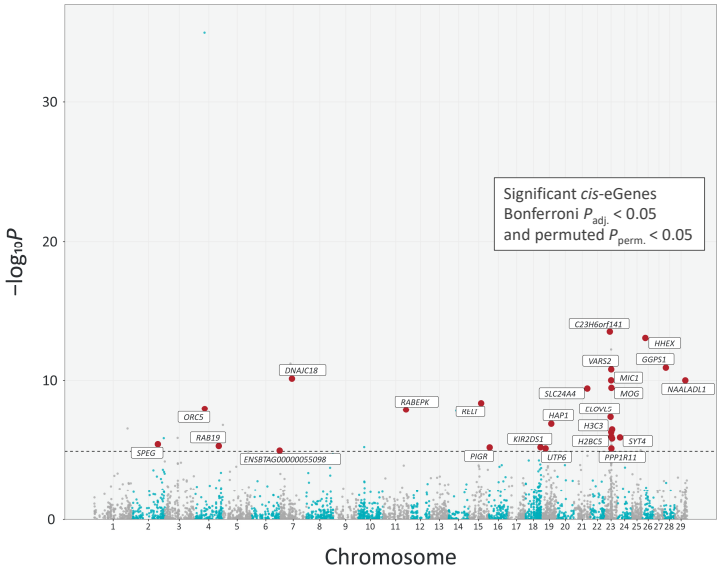


g:Profiler
https://biit.cs.ut.ee/gprofiler/

O'Grady J.F., *et al.* (2023) Peripheral blood transcriptomics and systems genetics of cattle infected with *Mycobacterium bovis* sheds light on the immunobiology of tuberculosis disease (in prep.).

10

Transcriptome-wide association study (TWAS) for bTB susceptibility in Holstein-Friesian cattle with *cis*-eGenes identified from an eQTL study using 3.9 million imputed SNPs

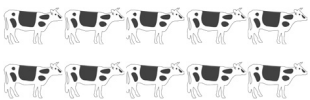


John O'Grady

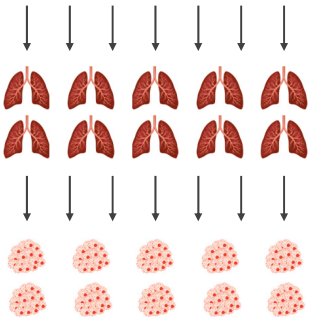
O'Grady J.F., *et al.* (in prep).
Ring S.C., *et al.* (2019) *PLoS ONE* **14**, e0212067, doi: 10.1371/journal.pone.0212067 (GWAS data set for TWAS integration).

11

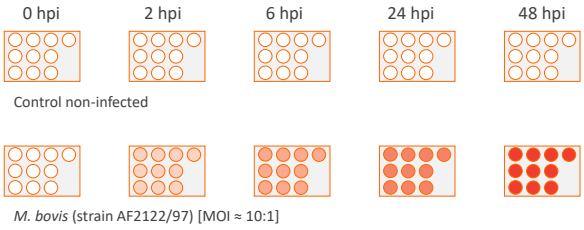
Bovine alveolar macrophage *in vitro* infection experiment



7-12 week-old H-F male calves



Alveolar macrophages (bAM)

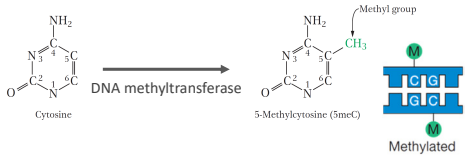


RNA-seq (transcriptomics – gene expression)
WGBS (genome-wide DNA methylation)
ChIP-seq (histone modifications and chromatin configuration)
MicroRNA-seq (transcriptomics – microRNA expression)

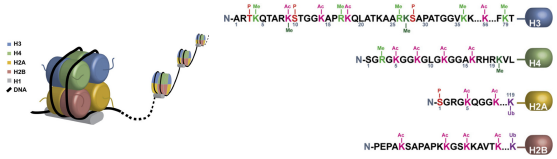
12

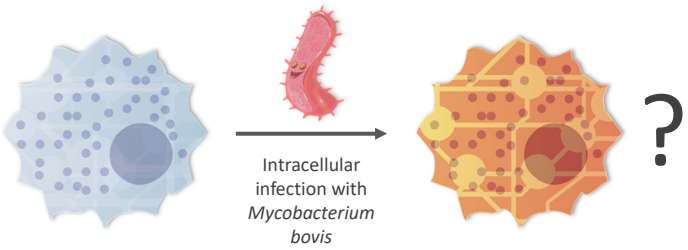
What role do epigenomic alterations (DNA methylation and histone modifications) have in reprogramming of the macrophage in response to mycobacterial infection?

DNA methylation (represses gene expression)



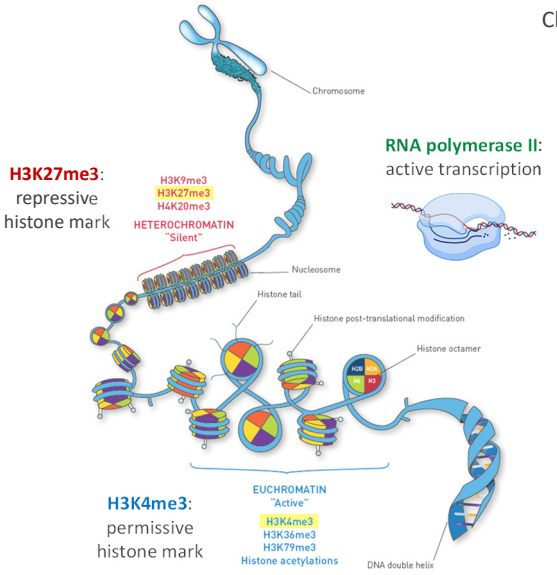
Histone modifications (can enhance or repress gene expression)



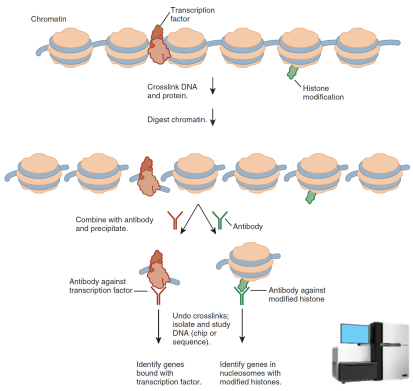


13

Investigation of chromatin modifications using ChIP-seq in bovine alveolar macrophages (bAM) infected with *Mycobacterium bovis*



Chromatin immunoprecipitation (ChIP) and sequencing (-seq)



Tom Hall

Alan O'Doherty

Doug Vernimmen

14

Alveolar macrophage chromatin is modified to orchestrate host response to *Mycobacterium bovis* infection

frontiers in Genetics

ORIGINAL RESEARCH
published: 07 February 2020
doi: 10.3389/fgene.2019.01386

Alveolar Macrophage Chromatin Is Modified to Orchestrate Host Response to *Mycobacterium bovis* Infection

Thomas J. Hall[1], Douglas Vernimmen[2], John A. Browne[1], Michael P. Mullen[3], Stephen V. Gordon[4,5], David E. MacHugh[1,6*] and Alan M. O'Doherty[1]*

[1] Animal Genomics Laboratory, UCD School of Agriculture and Food Science, College Dublin, Dublin, Ireland, [2] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, United Kingdom, [3] Bioscience Research Institute, Athlone Institute of Technology, Athlone, Ireland, [4] UCD School of Veterinary Medicine, University College Dublin, Dublin, Ireland, [5] UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

OPEN ACCESS

Edited by:
Jiuzhou Song,
University of Maryland,
College Park, United States

Reviewed by:
Luyang Sun,
Baylor College of Medicine,
United States
Ying Yu,
China Agricultural University
(CAU), China

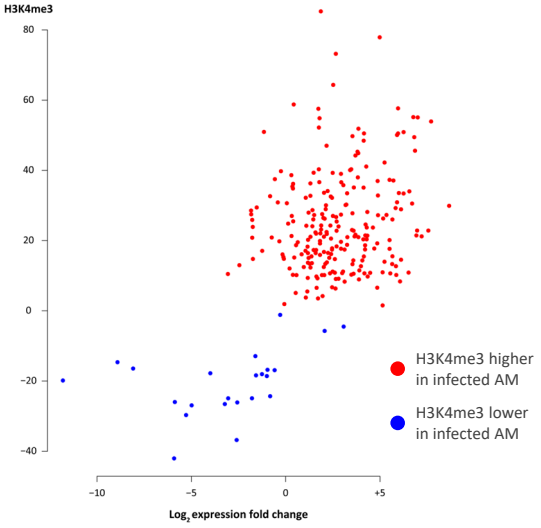
****Correspondence:***
Douglas Vernimmen
douglas.vernimmen@roslin.ed.ac.uk
David E. MacHugh
david.machugh@ucd.ie

Specialty section:
This article was submitted to Livestock Genomics, a section of the journal Frontiers in Genetics

Received: *06 June 2019*
Accepted: *18 December 2019*
Published: *07 February 2020*

Bovine tuberculosis is caused by infection with *Mycobacterium bovis*, which can also cause disease in a range of other mammals, including humans. Alveolar macrophages are the key immune effector cells that first encounter *M. bovis* and how the macrophage epigenome responds to mycobacterial pathogens is currently not well understood. Here, we have used chromatin immunoprecipitation sequencing (ChIP-seq), RNA-seq and miRNA-seq to examine the effect of *M. bovis* infection on the bovine alveolar macrophage (bAM) epigenome. We show that H3K4me3 is more prevalent, at a genome-wide level, in chromatin from *M. bovis*-infected bAM compared to control non-infected bAM; this was particularly evident at the transcriptional start sites of genes that determine programmed macrophage responses to mycobacterial infection (e.g. M1/M2 macrophage polarisation). This pattern was also supported by the distribution of RNA Polymerase II (Pol II) ChIP-seq results, which highlighted significantly increased transcriptional activity at genes demarcated by permissive chromatin. Identification of these genes enabled integration of high-density genome-wide association study (GWAS) data, which revealed genomic regions associated with resilience to infection with *M. bovis* in cattle. Through integration of these data, we show that bAM transcriptional reprogramming occurs through differential distribution of H3K4me3 and Pol II at key immune genes. Furthermore, this subset of genes can be used to prioritise genomic variants from a relevant GWAS data set.

H3K4me3: permissive histone mark



RNA-seq (gene expression)

Hall T.J., *et al.* (2020) *Front. Genet.* **10**, 1386. DOI: 10.3389/fgene.2019.01386

15

Alveolar macrophage chromatin is modified to orchestrate host response to *Mycobacterium bovis* infection

frontiers in Genetics

ORIGINAL RESEARCH
published: 07 February 2020
doi: 10.3389/fgene.2019.01386

Alveolar Macrophage Chromatin Is Modified to Orchestrate Host Response to *Mycobacterium bovis* Infection

Thomas J. Hall[1], Douglas Vernimmen[2], John A. Browne[1], Michael P. Mullen[3], Stephen V. Gordon[4,5], David E. MacHugh[1,6*] and Alan M. O'Doherty[1]*

[1] Animal Genomics Laboratory, UCD School of Agriculture and Food Science, College Dublin, Dublin, Ireland, [2] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, United Kingdom, [3] Bioscience Research Institute, Athlone Institute of Technology, Athlone, Ireland, [4] UCD School of Veterinary Medicine, University College Dublin, Dublin, Ireland, [5] UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

OPEN ACCESS

Edited by:
Jiuzhou Song,
University of Maryland,
College Park, United States

Reviewed by:
Luyang Sun,
Baylor College of Medicine,
United States
Ying Yu,
China Agricultural University
(CAU), China

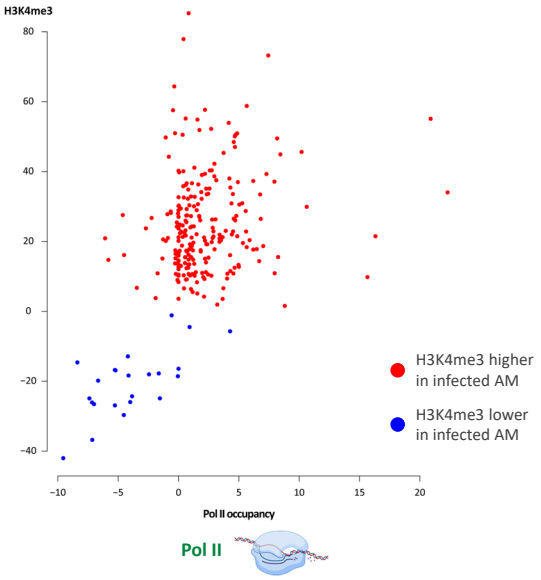
****Correspondence:***
Douglas Vernimmen
douglas.vernimmen@roslin.ed.ac.uk
David E. MacHugh
david.machugh@ucd.ie

Specialty section:
This article was submitted to Livestock Genomics, a section of the journal Frontiers in Genetics

Received: *06 June 2019*
Accepted: *18 December 2019*
Published: *07 February 2020*

Bovine tuberculosis is caused by infection with *Mycobacterium bovis*, which can also cause disease in a range of other mammals, including humans. Alveolar macrophages are the key immune effector cells that first encounter *M. bovis* and how the macrophage epigenome responds to mycobacterial pathogens is currently not well understood. Here, we have used chromatin immunoprecipitation sequencing (ChIP-seq), RNA-seq and miRNA-seq to examine the effect of *M. bovis* infection on the bovine alveolar macrophage (bAM) epigenome. We show that H3K4me3 is more prevalent, at a genome-wide level, in chromatin from *M. bovis*-infected bAM compared to control non-infected bAM; this was particularly evident at the transcriptional start sites of genes that determine programmed macrophage responses to mycobacterial infection (e.g. M1/M2 macrophage polarisation). This pattern was also supported by the distribution of RNA Polymerase II (Pol II) ChIP-seq results, which highlighted significantly increased transcriptional activity at genes demarcated by permissive chromatin. Identification of these genes enabled integration of high-density genome-wide association study (GWAS) data, which revealed genomic regions associated with resilience to infection with *M. bovis* in cattle. Through integration of these data, we show that bAM transcriptional reprogramming occurs through differential distribution of H3K4me3 and Pol II at key immune genes. Furthermore, this subset of genes can be used to prioritise genomic variants from a relevant GWAS data set.

H3K4me3: permissive histone mark



Hall T.J., *et al.* (2020) *Front. Genet.* **10**, 1386. DOI: 10.3389/fgene.2019.01386

16

Alveolar macrophage chromatin is modified to orchestrate host response to *Mycobacterium bovis* infection

frontiers in Genetics

ORIGINAL RESEARCH
published: 07 February 2020
doi: 10.3389/fgene.2019.01386

Alveolar Macrophage Chromatin Is Modified to Orchestrate Host Response to *Mycobacterium bovis* Infection

Thomas J. Hall[1], Douglas Vernimmen[2], John A. Browne[1], Michael P. Mullen[3], Stephen V. Gordon[4,5], David E. MacHugh[1,6*] and Alan M. O'Doherty[1]*

[1] Animal Genomics Laboratory, UCD School of Agriculture and Food Science, College Dublin, Dublin, Ireland, [2] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, United Kingdom, [3] Bioscience Research Institute, Athlone Institute of Technology, Athlone, Ireland, [4] UCD School of Veterinary Medicine, University College Dublin, Dublin, Ireland, [5] UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

OPEN ACCESS

Edited by:
Jiuzhou Song,
University of Maryland,
College Park, United States

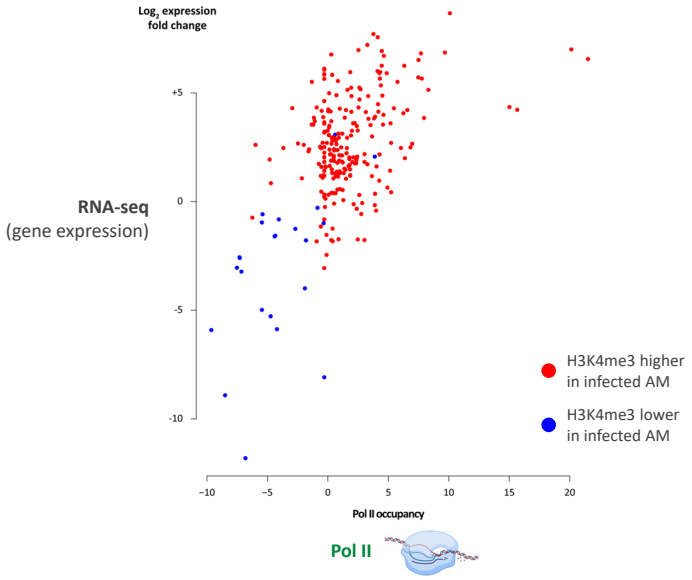
Reviewed by:
Luqing Sun,
Baylor College of Medicine,
Houston, United States
Ying Yu,
China Agricultural University
(CAU), China

***Correspondence:**
Douglas Vernimmen
douglas.vernimmen@roslin.ed.ac.uk
David E. MacHugh
david.machugh@ucd.ie

Specialty section:
This article was submitted to Livestock Genomics, a section of the journal Frontiers in Genetics

Received: *06 June 2019*
Accepted: *18 December 2019*
Published: *07 February 2020*

Bovine tuberculosis is caused by infection with *Mycobacterium bovis*, which can also cause disease in a range of other mammals, including humans. Alveolar macrophages are the key immune effector cells that first encounter *M. bovis* and how the macrophage epigenome responds to mycobacterial pathogens is currently not well understood. Here, we have used chromatin immunoprecipitation sequencing (ChIP-seq), RNA-seq and miRNA-seq to examine the effect of *M. bovis* infection on the bovine alveolar macrophage (bAM) epigenome. We show that H3K4me3 is more prevalent, at a genome-wide level, in chromatin from *M. bovis*-infected bAM compared to control non-infected bAM; this was particularly evident at the transcriptional start sites of genes that determine programmed macrophage responses to mycobacterial infection (e.g. M1/M2 macrophage polarisation). This pattern was also supported by the distribution of RNA Polymerase II (Pol II) ChIP-seq results, which highlighted significantly increased transcriptional activity at genes demarcated by permissive chromatin. Identification of these genes enabled integration of high-density genome-wide association study (GWAS) data, which revealed genomic regions associated with resilience to infection with *M. bovis* in cattle. Through integration of these data, we show that bAM transcriptional reprogramming occurs through differential distribution of H3K4me3 and Pol II at key immune genes. Furthermore, this subset of genes can be used to prioritise genomic variants from a relevant GWAS data set.



Hall T.J., *et al.* (2020) *Front. Genet.* **10**, 1386. DOI: 10.3389/fgene.2019.01386

17

ARG2 H3K4me3 ChiP-seq results: modulation by the pathogen?

H3K27me3: *M. bovis*-infected

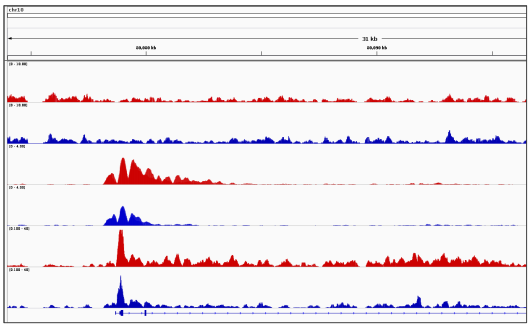
H3K27me3: Control non-infected

H3K4me3: *M. bovis*-infected

H3K4me3: Control non-infected

RNA Pol II: *M. bovis*-infected

RNA Pol II: Control non-infected



***ARG2* RNA-seq results:** Upregulated 10.7-fold in *M. bovis*-infected cells (FDR $P_{adj} = 7.52 \times 10^{-16}$)

Immune Evasion by *Helicobacter pylori* Is Mediated by Induction of Macrophage Arginase II

Nuruddeen D. Lewis,[e,†] Mohammad Asim,[e,‡] Daniel P. Barry,[e,‡] Thibaut de Sablet,[e,‡] Kshipra Singh,[e,‡] M. Blanca Piazuelo,[e] Alain P. Gobert,[e,1,2] Rupesh Chaturvedi,[e,2] and Keith T. Wilson[e,1,2]

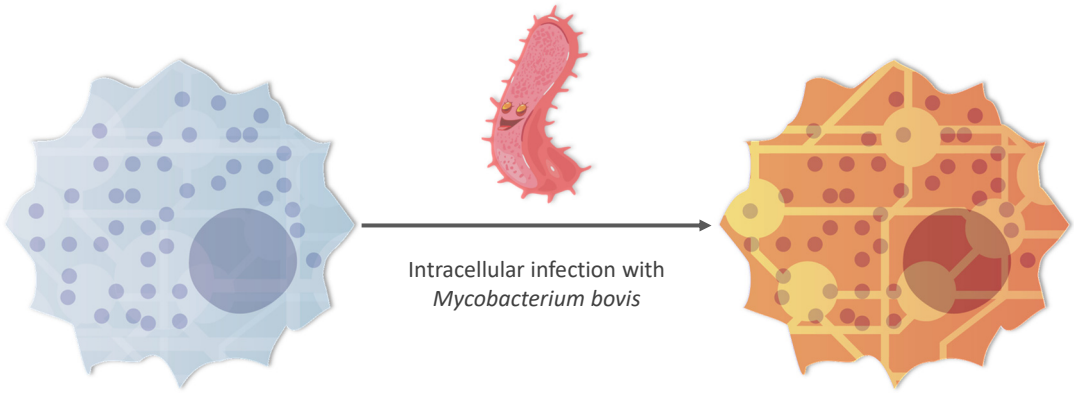
Arginase 2 deletion leads to enhanced M1 macrophage activation and upregulated polyamine metabolism in response to *Helicobacter pylori* infection

Dana M. Hardbower[1,2] · Mohammad Asim[2] · Tracy Murray-Stewart[1] · Robert A. Casero Jr.[1] · Thomas Verriere[2] · Nuruddeen D. Lewis[2] · Rupesh Chaturvedi[2,4] · M. Blanca Piazuelo[2] · Keith T. Wilson[1,2,5,6,7]

Hall T.J., *et al.* (2020) *Front. Genet.* **10**, 1386. DOI: 10.3389/fgene.2019.01386

18

What role do epigenomic alterations (DNA methylation and histone modifications) have in reprogramming of the macrophage in response to mycobacterial infection?



Reprogramming of the alveolar macrophage at 24 hpi is determined by histone modifications and chromatin remodelling.

19

Integrative genomics of the mammalian alveolar macrophage response to intracellular mycobacteria

Hall *et al. BMC Genomics* (2021) 22:343
https://doi.org/10.1186/s12864-021-07643-w

BMC Genomics

RESEARCH ARTICLE Open Access

Integrative genomics of the mammalian alveolar macrophage response to intracellular mycobacteria

Thomas J. Hall[1], Michael P. Mullen[2], Gillian P. McHugo[1], Kate E. Killick[1,3], Siobhán C. Ring[4], Donagh P. Berry[5], Carolina N. Correia[1], John A. Browne[1], Stephen V. Gordon[6,7] and David E. MacHugh[1,2*]

Abstract

Background: Bovine TB (bTB), caused by infection with *Mycobacterium bovis*, is a major endemic disease affecting global cattle production. The key innate immune cell that first encounters the pathogen is the alveolar macrophage, previously shown to be substantially reprogrammed during intracellular infection by the pathogen. Here we use differential expression, and correlation- and interaction-based network approaches to analyse the host response to infection with *M. bovis* at the transcriptome level to identify core infection response pathways and gene modules. These outputs were then integrated with genome-wide association study (GWAS) data sets to enhance detection of genomic variants for susceptibility/resistance to *M. bovis* infection.

Results: The host gene expression data consisted of RNA-seq data from bovine alveolar macrophages (bAM) infected with *M. bovis* at 24 and 48 h post-infection (hpi) compared to non-infected control bAM. These RNA-seq data were analysed using three distinct computational pipelines to produce six separate gene sets: 1) DE genes filtered using stringent fold-change and *P*-value thresholds (DEG-24: 378 genes, DEG-48: 390 genes); 2) genes obtained from expression correlation networks (CON-24: 460 genes, CON-48: 416 genes); and 3) genes obtained from differential expression networks (DEN-24: 339 genes, DEN-48: 495 genes). These six gene sets were integrated with three bTB breed GWAS data sets by employing a new genomics data integration tool—*gwinteR*. Using GWAS summary statistics, this methodology enabled detection of 36, 102 and 921 prioritised SNPs for Charolais, Limousin and Holstein-Friesian, respectively.

(Continued on next page)

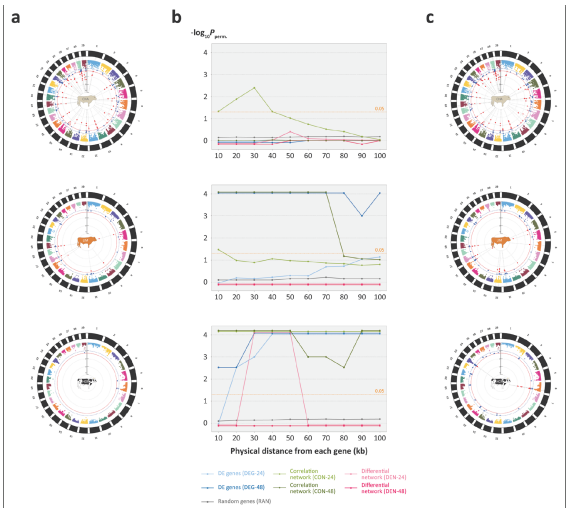


Fig. 5 Integration of bAM functional genomics and GWAS data for resistance to *M. bovis* infection in three cattle breeds. a Circular Manhattan plots showing GWAS results pre-integration with blue and red data points indicating binned SNP clusters with FDR $P_{adj.} < 0.10$ and < 0.05, respectively. b Line plots of permuted *P*-values across different genomic intervals for SNPs from six different input gene sets. c Circular Manhattan plots showing GWAS results post-integration using *gwinteR* with blue and red data points indicating binned SNP clusters with FDR $P_{adj.} < 0.10$ and < 0.05, respectively

Hall T.J., *et al.* (2021) *BMC Genomics* **22**, 343, DOI: 10.1186/s12864-021-07643-w.
Ring S.C., *et al.* (2019) *PLoS ONE* **14**, e0212067, doi: 10.1371/journal.pone.0212067 (GWAS data sets).

20

Integrative genomics of the mammalian alveolar macrophage response to intracellular mycobacteria

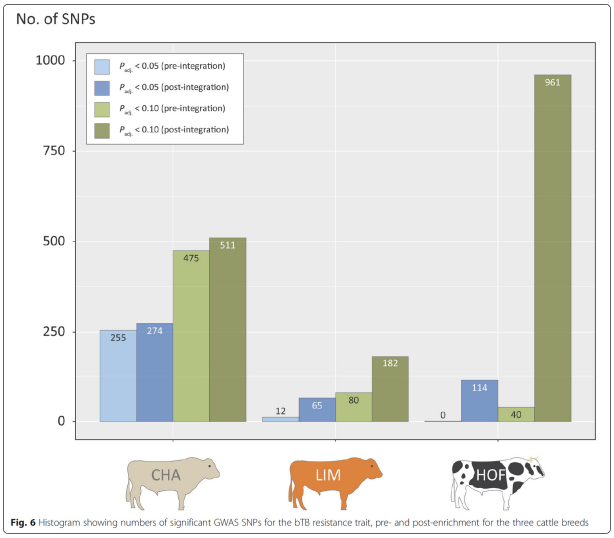


Fig. 6 Histogram showing numbers of significant GWAS SNPs for the bTB resistance trait, pre- and post-enrichment for the three cattle breeds

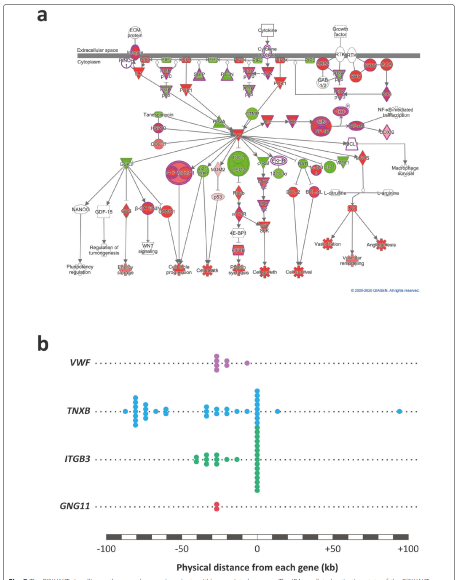
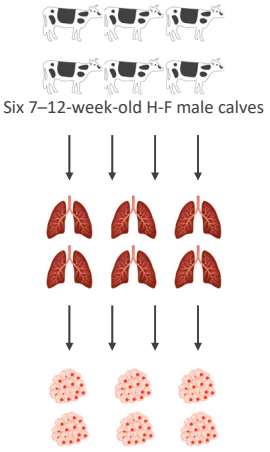


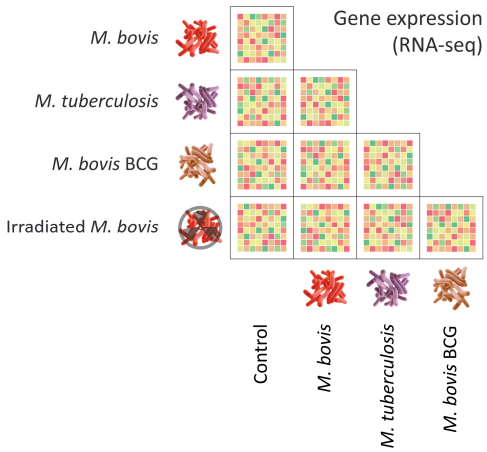
Fig. 7 The PI3K/AKT signalling pathway and genomic variants within associated genes. **a** The IPA-predicted activation state of the PI3K/AKT signalling pathway with red and blue colours indicating increased and decreased activity of pathway components, respectively. **b** Schematic of enriched bTB disease resistance GWAS SNPs in four genes associated with the PI3K/AKT signalling pathway

Hall T.J., *et al.* (2021) *BMC Genomics* **22**, 343, DOI: 10.1186/s12864-021-07643-w.
Ring S.C., *et al.* (2019) *PLoS ONE* **14**, e0212067, doi: 10.1371/journal.pone.0212067 (GWAS data sets).

21

Current work: using comparative multi-omics to understand the bovine alveolar macrophage response to host-adapted intracellular mycobacteria





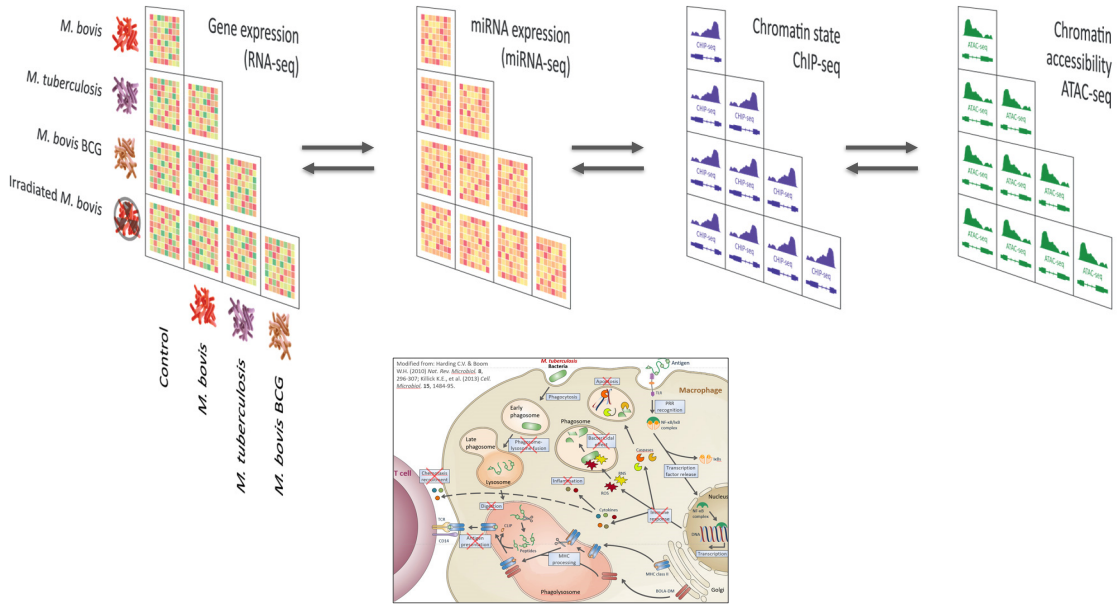
Tom Hall

Morgane Mitermite

John Browne

22

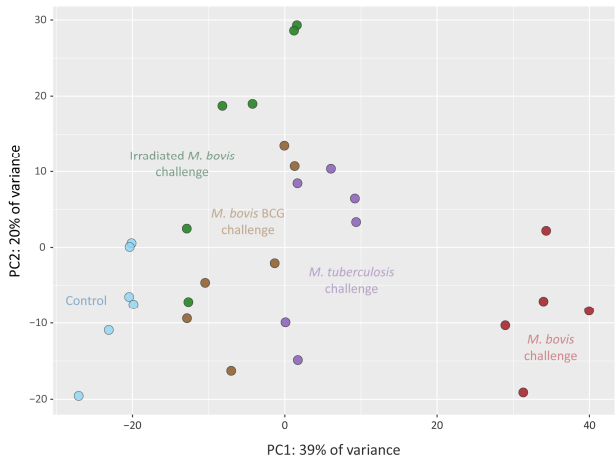
Current work: using comparative multi-omics to understand the bovine alveolar macrophage response to host-adapted intracellular mycobacteria



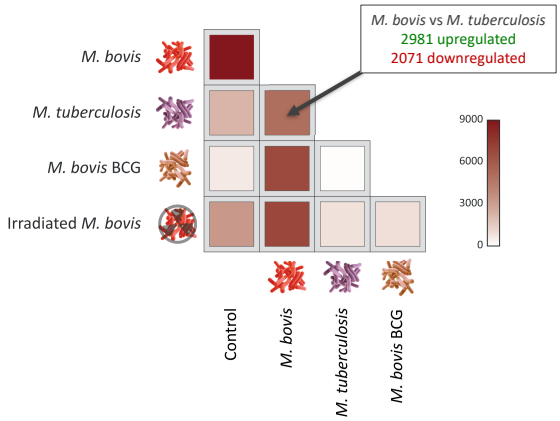
23

Preliminary gene expression (RNA-seq) results

Principal component analysis (PCA) of detectable gene expression



No. of differentially expressed genes from RNA-seq data
B-H FDR $P_{adj.} < 0.05$



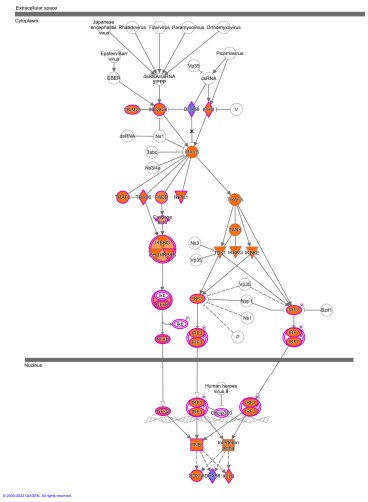
24

Preliminary gene expression (RNA-seq) pathway results

Ingenuity® Pathway Analysis (IPA®) results for *M. bovis* contrasts

Contrast	Cell type	All detectable genes	Analysis-ready genes	P_{adj} cut-off	Significant genes	Significant genes: upregulated	Significant genes: downregulated	Top five IPA pathways	*P* value	% overlap	Overlap
M. bovis vs Control	Macrophages	22,259	12,733	**0.00001**	2648	1658	990	Interferon Signaling	5.96E-12	78.1%	25/32
								FAT10 Signaling Pathway	9.39E-11	62.7%	32/51
								Activation of IRF by Cytosolic Pattern Recognition Receptors	1.73E-09	60.0%	30/50
								Death Receptor Signaling	1.50E-08	48.2%	41/85
								Protein Ubiquitination Pathway	1.95E-08	36.2%	87/240
M. bovis vs irradiated *M. bovis*	Macrophages	21,221	12,613	**0.001**	2883	1660	1223	Interferon Signaling	4.24E-08	68.8%	22/32
								Activation of IRF by Cytosolic Pattern Recognition Receptors	6.78E-06	52.0%	26/50
								Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	1.17E-05	50.0%	27/54
								Retinoic acid Mediated Apoptosis Signaling	2.20E-05	55.6%	20/36
								Role of RIG1-like Receptors in Antiviral Innate Immunity	2.52E-05	58.1%	18/31
M. bovis vs *M. bovis* BCG	Macrophages	21,205	12,611	**0.001**	P	1699	1093	Interferon Signaling	2.86E-10	75.0%	24/32
								Activation of IRF by Cytosolic Pattern Recognition Receptors	8.21E-09	60.0%	30/50
								Immunogenic Cell Death Signaling Pathway	4.96E-08	51.4%	37/72
								Role of RIG1-like Receptors in Antiviral Innate Immunity	7.15E-08	67.7%	21/31
								Inhibition of ARE-Mediated mRNA Degradation Pathway	2.54E-07	40.8%	60/147
M. bovis vs *M. tuberculosis*	Macrophages	20,183	12,456	**0.01**	2572	1727	1025	Interferon Signaling	1.61E-07	65.6%	21/32
								Immunogenic Cell Death Signaling Pathway	6.69E-06	45.8%	33/72
								Activation of IRF by Cytosolic Pattern Recognition Receptors	1.34E-05	50.0%	25/50
								Role of RIG1-like Receptors in Antiviral Innate Immunity	1.56E-05	58.1%	18/31
								Protein Ubiquitination Pathway 3.16E-05 33.5 % 80/239	1.56E-05	58.1%	18/31

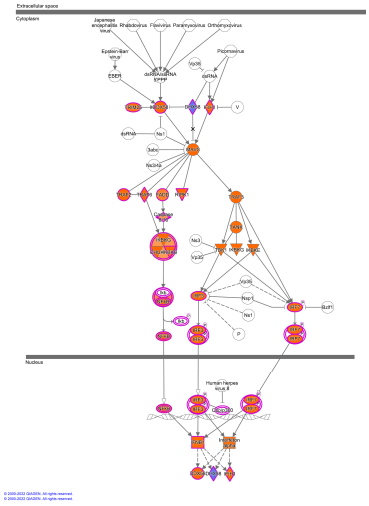
Role of RIG-I-like receptors in antiviral innate immunity (*M. bovis* vs *M. tuberculosis*)

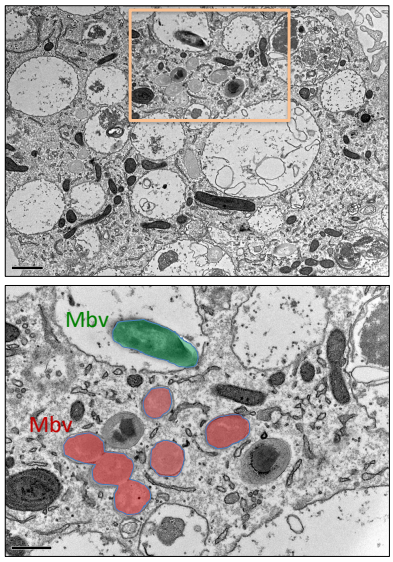


25

Bovine alveolar macrophages at 24 hpi: phagolysosomal escape by *M. bovis*

Role of RIG-I-like receptors in antiviral innate immunity (*M. bovis* vs *M. tuberculosis*)





M. bovis: membrane-bound vs cytosolic

Electron micrograph credit: Antony Fearns and Max Gutierrez

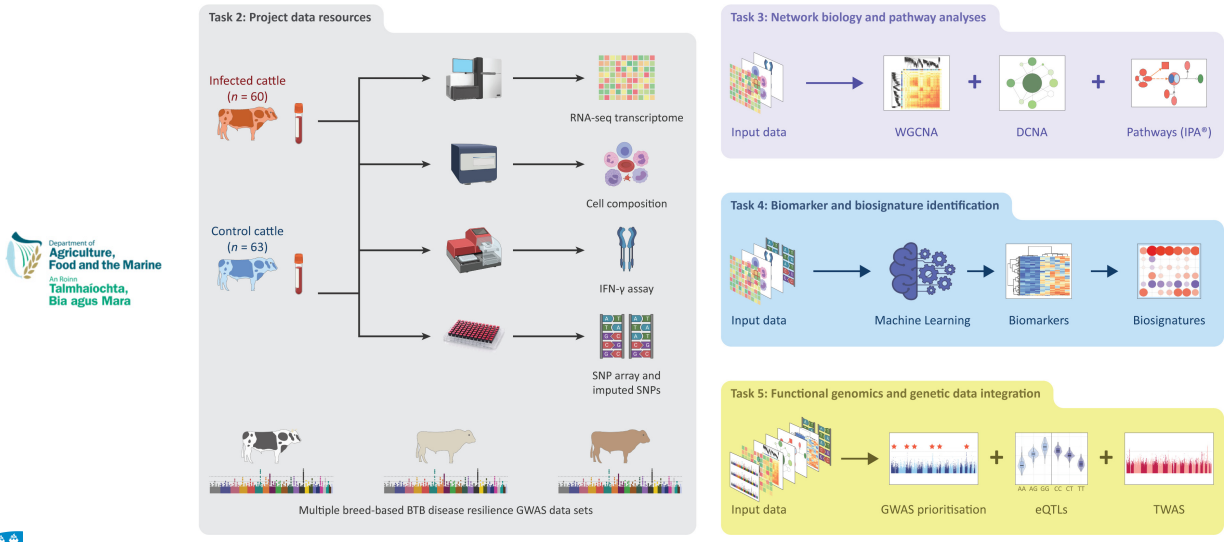
26

Current work: using comparative multi-omics to understand the bovine alveolar macrophage response to host-adapted intracellular mycobacteria

- A multi-omics approach is likely to be the best strategy for dissecting and teasing apart the molecular machinery that determines the reprogramming of alveolar macrophages in response to infection with MTBC pathogens that cause tuberculosis disease.
- Ultimately, we expect this approach will lead to an improved understanding of the factors that determine the host tropism and coevolution of MTBC pathogens and their vertebrate hosts.
- Integration of multi-omics-prioritized genes (510 genes) with large cattle GWAS data sets significantly improved detection of QTLs underlying a defined resistance to *M. bovis* infection trait (results not shown here).
- Future work will focus on leveraging our results to enhance biology-directed selection for disease resilience and identifying potential targets for genome editing.

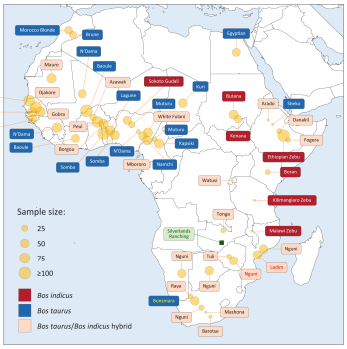
27

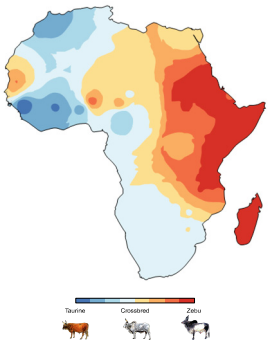
Integrative genomics and development of next-generation tools for the control and eradication of bovine tuberculosis (TB-ORNOT-TB)



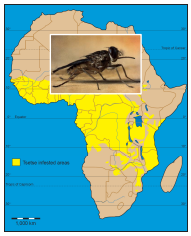
28

Population and integrative genomics of African cattle: a high-resolution window on mammalian microevolution





Adapted from Hanotte O., *et al.* (2002) African pastoralism: genetic imprints of origins and migrations. *Science* **296**, 336-9.



Dan Bradley

Trinity College Dublin

Laurent Frantz

Tad Sonstegard

acceligen

James Ward

Gillian McHugo

Ward J.A. *et al.* (2022) *iScience* **25**. DOI: 10.1016/j.isci.2022.104672

29

Acknowledgements, support and funding

Steve Gordon (UCD)
Thomas Hall (UCD)
John O'Grady (UCD)
John Browne (UCD)
Gillian McHugo (UCD)
Morgane Mitermite (UCD)
Alan O'Doherty (Novartis, ex-UCD)
Kerri Malone (EMBL-EBI, ex-UCD)
James Ward (UCD)
Alia Parveen (UCD)
Eamonn Gormley (UCD)
Kieran Meade (UCD)
Claire Gormley (UCD)
Carol Correia (HSE, ex-UCD)
Dave Magee (Novartis, ex-UCD)

TNicolas Nalpas (Tübingen University, ex-UCD)
ad Sonstegard (Acceligen/Recombinetics)
Douglas Vernimmen (Roslin Institute)
Kevin Conlon (Jannsen, ex-UCD)
Kate Killick (Novartis, ex-UCD)
Donagh Berry (Teagasc)
Siobhan Ring (ICBF)
Mike Mullen (Athlone IT)
Emily Clark (Roslin Institute)
Mazdak Salavati (Roslin Institute)
Max Gutierrez (Crick Institute)
Antony Fearns (Crick Institute)
Dan Bradley (TCD)
Laurent Frantz (LMU)

Science Foundation Ireland For what's next

Supported by wellcome trust
IRISH RESEARCH COUNCIL An Chomhairle um Thaighde in Éirinn
FAANG
Trinity College Dublin
ROSLIN
THE UNIVERSITY of EDINBURGH
LMU
SEVENTH FRAMEWORK PROGRAMME
cost EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY
SCIENCE
CAPES
ICBF.com
teagasc
Department of Agriculture, Food and the Marine
NDP

30