Next Generation Sequencing – The Role of New Sequence Technologies in Shaping the Future of Veterinary Science

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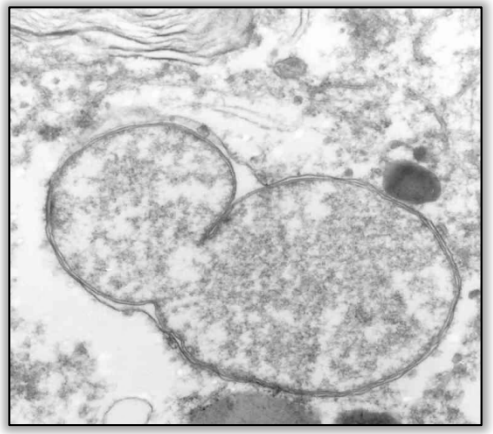


Wolbachia gene expression and River Blindness: a sinuous tale from comparative medicine

Alistair Darby, Stuart Armstrong, Germanus Bah, Vincent Tanya, Jonathan Wastling, Alexander Trees & **Benjamin Makepeace**

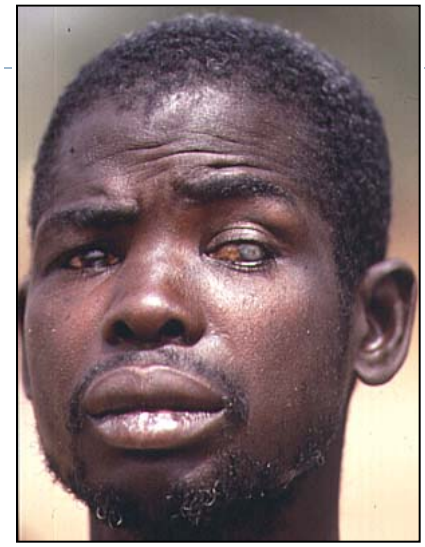
CENTRE for GENOMIC RESEARCH CGR

Onchocerca volvulus in humans

MACROFILARIAE

Subcutaneous nodules (scapulae, skull, iliac crest, knees, elbows)

Limited pathological significance



MICROFILARIAE

←Eyes

Skin↓

Pruritis, lichenification, premature aging, leopard skin, Sowda

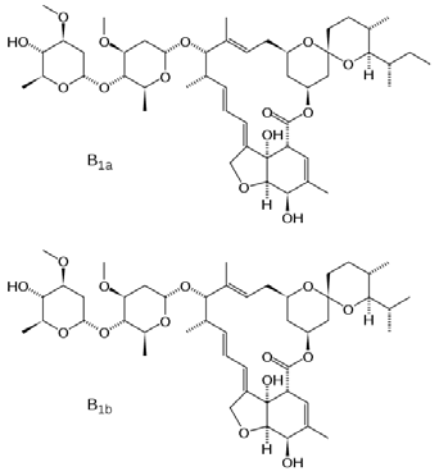
PREVALENCE (APOC, 2005):	**37 million infected in sub-Saharan Africa**
Disease burden (Mathers *et al.*, 2007):	**349,000 blind**
	601,000 severely visually disabled
	1,346,000 pruritis
Total	**2,296,000**

Onchocerca ochengi in cattle

- The closest relative of the human parasite
- Transmitted by the same group of insect vectors (*Simulium damnosum* complex)
- Adult parasites form collagenous nodules with comparable structure to human nodules
- Extremely similar antigenically
- Parallel response to drugs
- Contains *Wolbachia*

Is onchocerciasis eradicable in Africa?

- Transmission interrupted after 15 – 17 years
  - River Gambia focus, Senegal (semi-annual treatment)
  - River Bakoye focus, Mali (annual treatment)
- Transmission ongoing after 13 - 17 years
  - North Region, Cameroon (annual treatment)
  - Several districts in Uganda (annual treatment)
- Challenges with ivermectin
  - Lack of adulticidal effect
  - Ivermectin resistance – Ghana?
  - Severe adverse events in loiasis
  - Conflict (DRC, Sudan, Uganda)



A little serendipity…

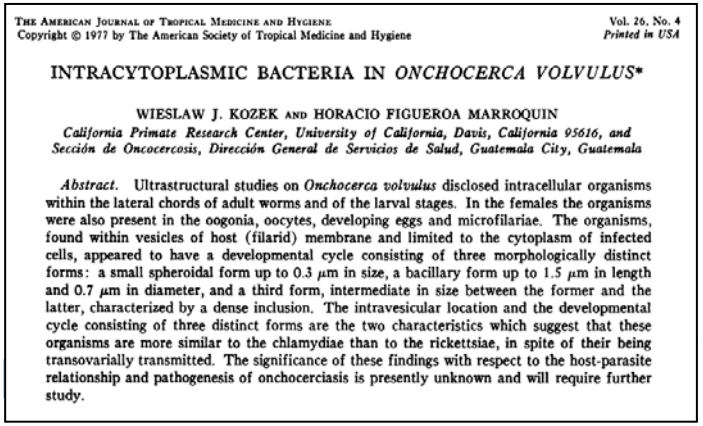
- A 'control' animal infected with *O. ochengi* developed a co-infection (dermatophilosis)
- Repeated treatments with oxytetracycline over 6 months led to incidental clearance of all nodules
- In parallel, research in rodent models of filariasis showed that antibiotics affected the worms' development and reproduction

Controlled trial of oxytetracycline

Table 1. *Effects of oxytetracycline treatment of cattle on* O. ochengi *worm number and motility*

	control				oxytetracycline			
	no. of worms[a]		motility[b] (median)		no. of worms		motility (median)	
months	female	male	female	male	female	male	female	male
0	12	12	2	2	13	20	2	2
1	12	6	2	2	12	24	2	2
3	12	24	2	2	12	17	2	2
6	12	23	2	2	12	7	0	0
9	12	9	2	2	2	2	0	0

[a]From 12 nodules or nodule sites.
[b]Classification of motility scores: 0, no movements; 1, slow, occasional movement; 2, normal vigorous movements after 30 min incubation at 36° C.



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INTRACYTOPLASMIC BACTERIA IN *ONCHOCERCA VOLVULUS**

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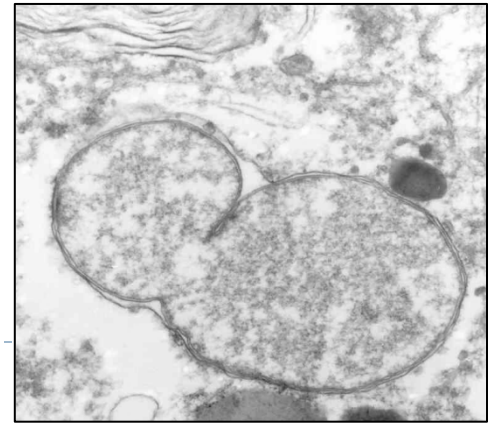
Abstract. Ultrastructural studies on *Onchocerca volvulus* disclosed intracellular organisms within the lateral chords of adult worms and of the larval stages. In the females the organisms were also present in the oogonia, oocytes, developing eggs and microfilariae. The organisms, found within vesicles of host (filarid) membrane and limited to the cytoplasm of infected cells, appeared to have a developmental cycle consisting of three morphologically distinct forms: a small spheroidal form up to 0.3 μm in size, a bacillary form up to 1.5 μm in length and 0.7 μm in diameter, and a third form, intermediate in size between the former and the latter, characterized by a dense inclusion. The intravesicular location and the developmental cycle consisting of three distinct forms are the two characteristics which suggest that these organisms are more similar to the chlamydiae than to the rickettsiae, in spite of their being transovarially transmitted. The significance of these findings with respect to the host-parasite relationship and pathogenesis of onchocerciasis is presently unknown and will require further study.



One giant leap for Lord Trees…

THE ROYAL SOCIETY

Macrofilaricidal activity of tetracycline against the filarial nematode *Onchocerca ochengi*: elimination of *Wolbachia* precedes worm death and suggests a dependent relationship

Nigel G. Langworthy[1], Alfons Renz[2], Ute Mackenstedt[2], Kim Henkle-Dührsen[3], Mark B. de C. Bronsvoort[1], Vincent N. Tanya[4], Martin J. Donnelly[1] and Alexander J. Trees[1]*

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RESEARCH LETTERS

3335 [594] g, n=106, p=0·014). Fetal T allele carrier status was not associated with a significantly lower birthweight (CC babies 3425 [623] g, n=51; CT and TT babies 3441 [556] g, n=62, p=0·87).

We showed that the maternal G protein β3 subunit (*825T*) allele in healthy normal pregnant women was associated with a lower birthweight. Given that birthweight is a multifactorial phenomenon, involving factors such as non-genetic socioeconomic circumstances, as well as maternal and fetal gene alterations, the association of the *825T* allele is striking. The molecular pathways that mediate an impaired fetal growth associated with this allele need to be clarified.

This study was supported by a research grant from the Universitätsklinikum Charité der Humboldt Universität zu Berlin. We thank Christine Lehmann for technical assistance.

1 Leon DA, Lithell HO, Vagero D, et al. Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915–29. *BMJ* 1998; **317**: 241–45.
2 Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 1991; **303**: 1019–22.
3 Siffert W, Rosskopf D, Siffert G, et al. Association of a human G-protein beta3 subunit variant with hypertension. *Nat Genet* 1998; **1**: 45–48.

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Endosymbiotic bacteria in worms as targets for a novel chemotherapy in filariasis

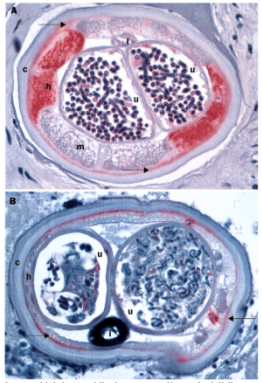
Achim Hoerauf, Lars Volkmann, Christoph Hamelmann, Ohene Adjei, Ingo B Autenrieth, Bernhard Fleischer, Dietrich W Büttner

Endosymbiotic bacteria living in plasmodia or worm parasites are required for the homoeostasis of their host and should be excellent targets for chemotherapy of certain parasitic diseases. We show that targeting of *Wolbachia* spp bacteria in *Onchocerca volvulus* filariae by doxycycline leads to sterility of adult worms to an extent not seen with drugs used against onchocerciasis, a leading cause of blindness in African countries.

Filariae are responsible for devastating diseases in man, including blindness and elephantiasis, with 150 million infections worldwide. The world community had made it a goal to interrupt transmission and to eliminate these diseases.[1] However, present chemotherapy[2] such as ivermectin (drug of first choice) are mainly targeted at mature microfilariae, and not at adult worms or early embryos, leading to a reappearance of skin microfilariae several months after treatment. Since adult worms have a long lifespan (up to 15 years), mass treatment will have to be maintained for many years if transmission is to be interrupted.[1] Computer simulation shows tremendous risks in these programmes with present drugs alone.[3] There is thus a pressing need for new antifilarial drugs that have microfilaricidal efficacy or that show total and longlasting suppression of embryo production, to complement microfilaricides such as ivermectin.[4]

Evidence from work in animals shows that *Wolbachia* spp (order Rickettsiales) endobacteria in filariae are targets for chemotherapy, since their depletion by tetracycline led to degeneration and sterility of adult worms.[5,6] This approach has not been examined in human filariasis. Therefore, we investigated the effectiveness of targeting wolbachia in human onchocerciasis with respect to worm fertility and survival.

In an area of Ghana outside the onchocerciasis control programme, volunteer onchocerciasis patients aged 18–50 years who had not had ivermectin were assigned, after informed consent, to a control group or to treatment with doxycycline (Vibramycin, Pfizer; 100 mg orally per day) for 6 weeks. Daily tablet intake was supervised. 4 months after the end of treatment, which was well tolerated in all cases, onchocercomata (nodules containing one to six female worms) were excised and coded for blinded examinations by two independent examiners. One part of each nodule was analysed by immunohistology for the presence of wolbachia and for morphological alterations (ie, adult worm degeneration, disturbance of embryogenesis, table; methods as described earlier[7]). The other part was processed for semiquantitative PCR to quantify bacterial



Immunohistology: midbody cross-sections of adult live *Onchocerca volvulus* filariae
APAAP method was used, with rabbit antiserum against bacterial hsp-60. (A) Worm from control (no doxycycline). Numerous bacteria are stained as corpuscular red bodies in the hypodermal cords (h), but are not seen in the cuticle (c), the musculature (m), the intestine (i), and the uterus epithelium (u). Arrows=non-corpuscular, less intense staining, possibly of nematode hsp-60, in the areas of known high mitochondrial density. Uteri show normal embryogenesis with stretched microfilariae: ×180.
(B) Worm from patient on doxycycline. No bacteria are detectable in hypodermis. Non-corpuscular straining in areas of mitochondrial density (arrows). Embryos in uteri are clearly degenerated, with pretzel stages showing irregular body shape: ×135.

Antibiotic Chemotherapy of Onchocerciasis: In a Bovine Model, Killing of Adult Parasites Requires a Sustained Depletion of Endosymbiotic Bacteria (*Wolbachia* Species)

The Journal of Infectious Diseases 2005; 192:1483–93

Jeffrey Gilbert,[1,a] Charles K. Nfon,[1,2,a,b] Benjamin L. Makepeace,[1] Leo M. Njongmeta,[1,b] Ian M. Hastings,[1] Kenneth M. Pfarr,[3] Alfons Renz,[4] Vincent N. Tanya,[2] and Alexander J. Trees[1]

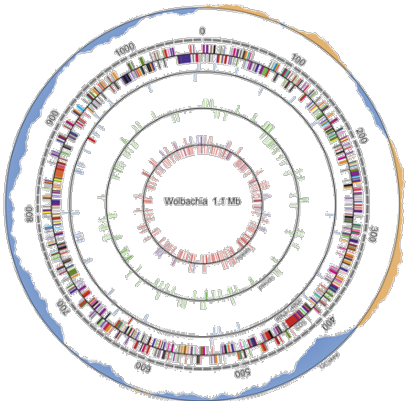
[1]Liverpool School of Tropical Medicine and Faculty of Veterinary Science, University of Liverpool, United Kingdom; [2]Institut de Recherche Agricole pour le Développement, Wakwa, Cameroon; [3]Institute for Medical Parasitology, University of Bonn, Germany, [4]Institute for Animal Physiology, University of Tübingen, Germany

Wolbachia: background

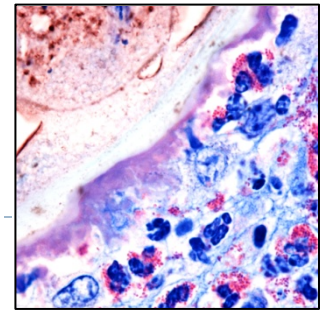
- Obligate intracellular bacterium related to rickettsiae
- Probably the most prevalent vertically-transmitted symbiont on Earth
  - An estimated 40% of arthropods are infected
  - Much more limited distribution in parasitic nematodes (some filariae, and a parasite of banana plants!)
- Diverse effects on their hosts
  - Reproductive parasite in many arthropods; *e.g.* sex-ratio biases
  - Mutualist: provision of nutrients, pathogen protection, immune evasion

What is the basis of the symbiosis?

- Genome sequence of *Wolbachia* from *Brugia malayi* (*w*Bm) suggests that it may provide nutrients to the worm host (Foster *et al*., 2005):
  - Haem
  - Riboflavin
  - Flavin adenine dinucleotide
  - Nucleotides

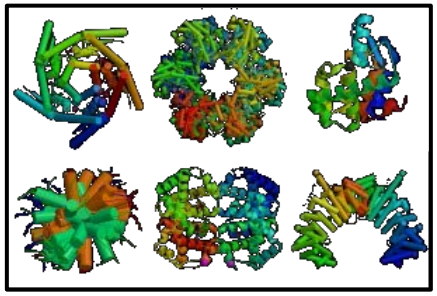


- In onchocerciasis, *Wolbachia* attracts neutrophils around adult worms:
  - Antibiotic treatment leads to clearance of neutrophilia, followed by eosinophil infiltration and death of adult worms (Hansen *et al*., 2011)
  - Suggests that *Wolbachia* is a 'defensive mutualist'



Methods

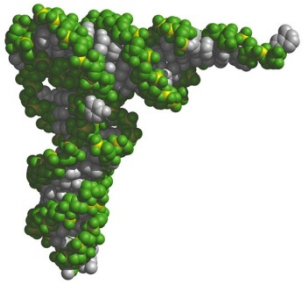
Proteins



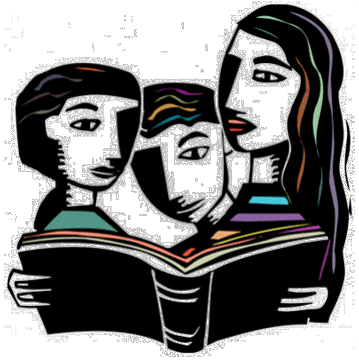
Proteomics

What is the organism made of?

RNA



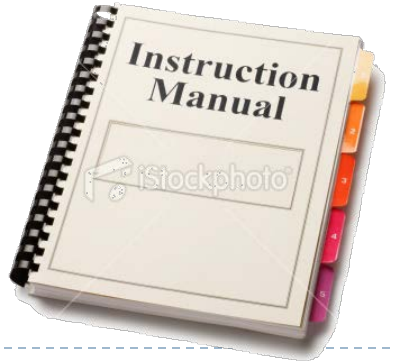
Transcriptomics



Which instructions are "read" at what time?

DNA

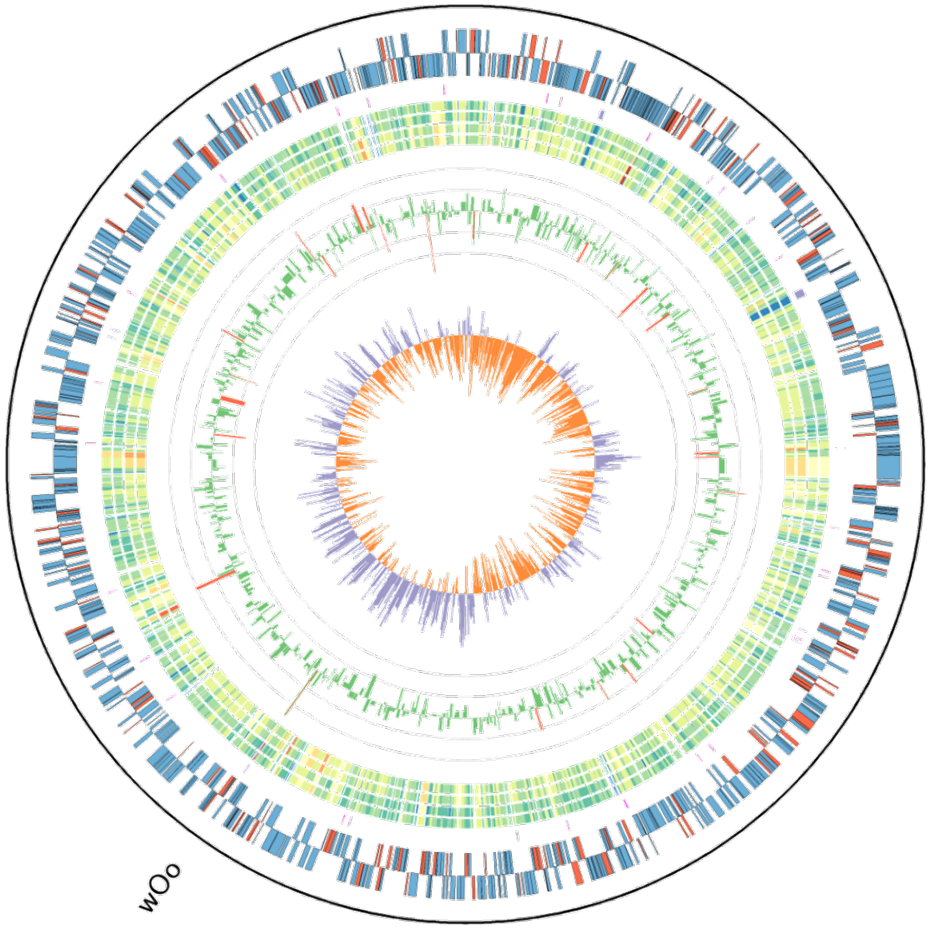
Genomics



What do the organism's instructions say?

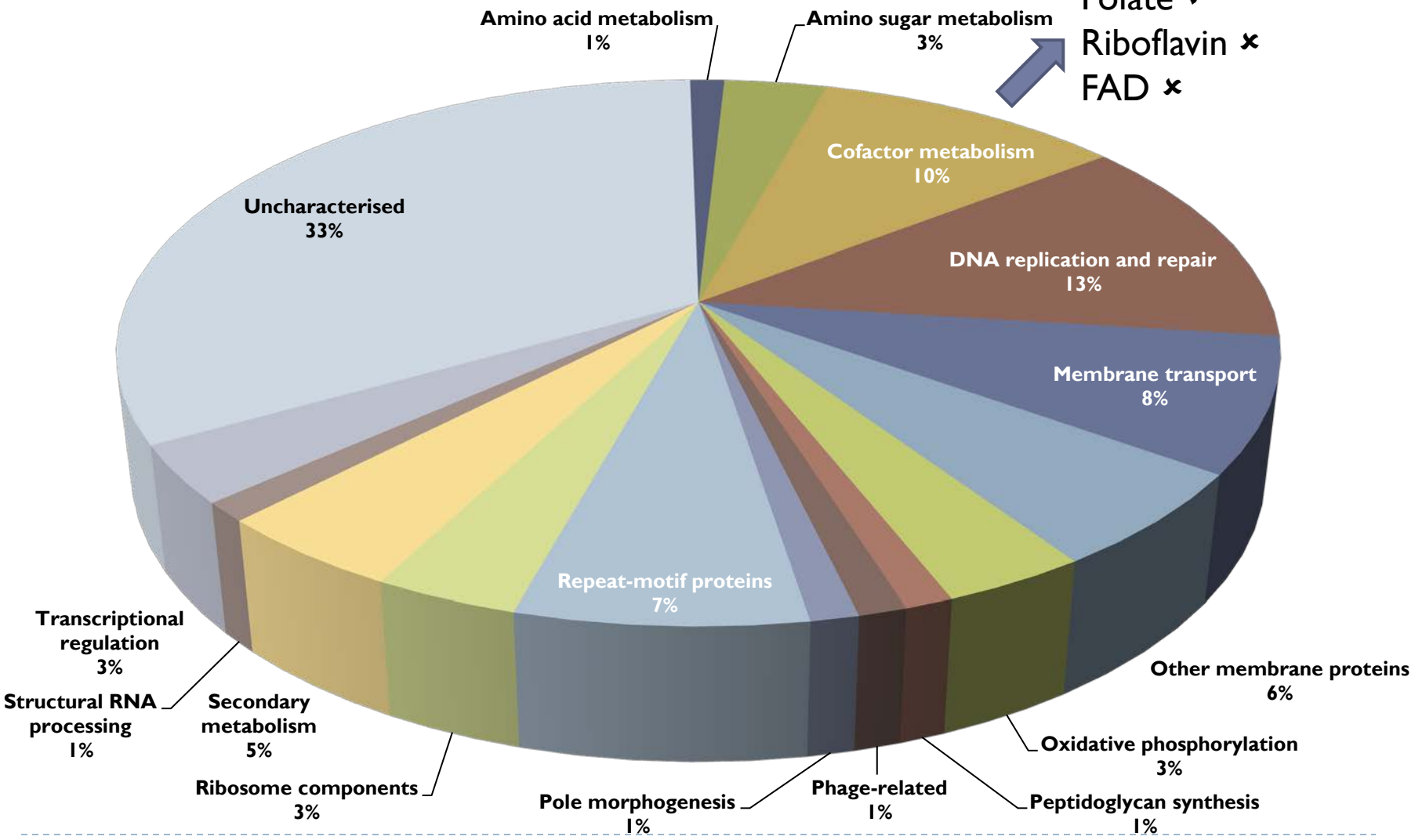
The *Wolbachia* genome of *O. ochengi*

- The first *Wolbachia* genome from its clade and the smallest sequenced to date
  - 11% smaller than *w*Bm
  - 160 fewer intact genes than *w*Bm



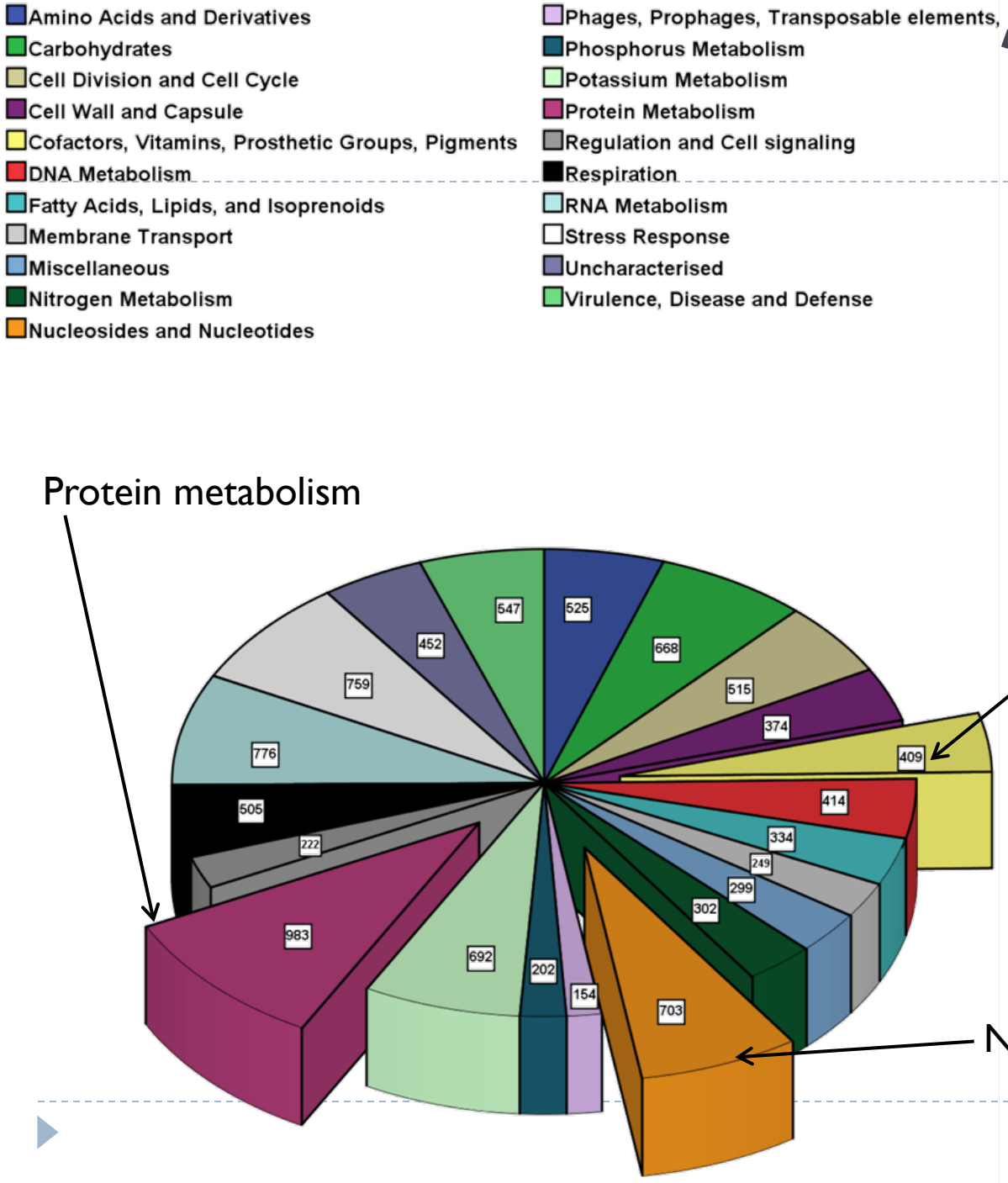
Gene losses

Haem ✓
Folate ✓
Riboflavin ✗
FAD ✗



n = 88

Transcriptome overview



Differential transcription: soma/gonad

- Soma
  - Membrane transport
    - VirB2
    - Major facilitator superfamily permease
    - Multiple resistance/pH regulation protein
    - Cation diffusion facilitator
  - Respiration
    - Cytochrome *c* oxidase (subunit III)
    - Thiol-disulfide isomerase

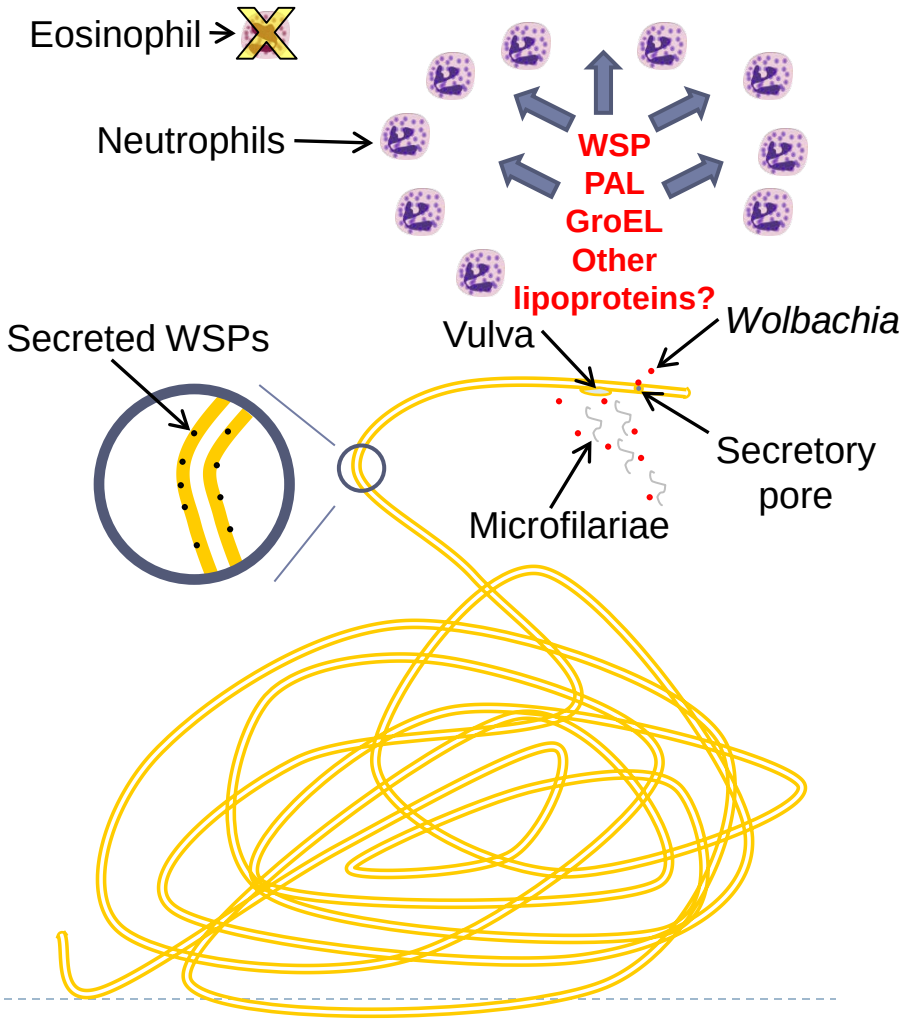
- Gonad
  - Translation
    - Elongation factor Tu
    - Several ribosomal proteins
    - Polypeptide deformylase
  - DNA replication
    - Uracil-DNA glycosylase
    - HU nucleoid protein
    - Secreted nuclease Nuc
  - Membrane transport
    - Preprotein translocase subunit SecF
    - Signal peptidase I
  - Respiration
    - F_oF_1-type ATP synthase (δ subunit)

Expression of abundant proteins

Protein	No. unique peptides	Function
Chaperonin GroEL	45	Protein processing
Outer membrane protein	26	Membrane transport
ATP-binding subunit Clp protease	25	Protein processing
Wolbachia surface protein (WSP)	17	Interactions with host
Hypothetical protein WPa 0007	14	Unknown
Hypothetical protein WPa 0828	11	Unknown
Peptidoglycan-associated lipoprotein	9	Cell envelope stabilisation
Molecular chaperone DnaK	9	Protein processing
Putative modulator of DNA gyrase	8	Protein processing
Succinyl-CoA synthetase, β subunit	8	Energy production
F-type H^+-transporting ATPase, subunit β	7	Energy production
Fructose-bisphosphate aldolase	7	Nucleotide synthesis

Wolbachia: conclusions

- Metabolic role
  - 'Supplementary mitochondrion' → ATP production
  - Recycling and detoxification of iron?
- Immune defence
  - Stimulation of antibacterial (neutrophil) response
    - *Wolbachia* surface protein (WSP), heat-shock protein GroEL, peptidoglycan-associated lipoprotein (PAL)
  - Inhibition of eosinophil effector mechanisms



Acknowledgments

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  - Alan Radford, Neil Hall *et al.*
- Additional genome reads for sequence assembly
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