Manganese: a key regulator of global cell physiology?

Alastair McEwan & Michael Jennings

School of Molecular and Microbial Sciences
The University of Queensland
E-mail: mcewan@uq.edu.au

Iron is recognised as central element in bacterial physiology and the control of the iron acquisition regulon in relation to iron status is an established example of how a metal ion can influence gene expression [1]. In contrast, manganese is an element that has long been known to be an essential 'trace element' but, until recently, its influence on gene expression and microbial cell physiology was largely overlooked.

In this short review we will present the evidence that manganese may be of major importance in the regulation of gene expression, especially in relation to oxidative stress and close by highlighting evidence that is outside the scope of this article that indicates that manganese is a transition metal of major importance in bacterial physiology.

The contrasting redox chemistry of manganese and iron

Iron is the key metal ion in a wide variety of redox reactions in the cell as a component of haem groups, iron-sulfur clusters and in a variety of non-haem iron centres. The oxidation-reduction potential of free Fe^{2+}/Fe^{3+} states is +0.77mV but this can be modulated greatly by the coordination environment of the iron. In biological systems, ferrous iron is a sufficiently strong electron donor for it to be reactive towards the reactive oxygen species hydrogen peroxide:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^-$$

This is the Fenton reaction which leads to the generation of the hydroxyl radical, a powerful oxidising agent which damages a variety of cellular components. The relatively high redox potential of the Fe^{2+}/Fe^{3+} redox couple means that Fe^{3+} is easily reduced within the cell by molecules such as superoxide:

$$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2$$

When the two reactions are combined, we arrive at iron-catalysed formation of hydroxyl radical from superoxide and hydrogen peroxide, the Haber-Weiss reaction. As a consequence of its pro-oxidant properties, iron needs to be handled with great care by cells.

Contrast the above situation with that of manganese – the oxidation-reduction potential of the free Mn^{2+}/Mn^{3+} states is +1.51mV. This is too high for free Mn^{2+} to react with hydrogen peroxide and generate hydroxyl radicals. However, Mn^{3+} species is a very strong electron acceptor and this makes manganese an excellent antioxidant. Thus, although manganese and iron are adjacent in the periodic table, their chemistry has quite a different impact on cellular oxidative stress [2].

Manganese transport and defence against oxidative stress

Given the toxicity of iron, the question is, why use it if it can be avoided? Iron is a wonderful catalyst and is especially useful in reactions that make use of oxygen but, if a microbe does not respire and can make a living by fermentation, then why use such a toxic element? The lactic acid bacteria carry out fermentation and have no respiratory chain and, as a consequence, they have a very limited requirement for iron. However, unlike obligate anaerobes, these organisms are aerotolerant and, as a consequence, are subject to oxidative stress. Accumulation of manganese by these bacteria may be central to defence against oxidative stress. The case of *Lactobacillus plantarum* provides a notable example of the use of manganese as an antioxidant and there is clear evidence that, in this bacterium, which lacks superoxide dismutases, manganese acts as a chemical quencher of superoxide.

In contrast to *L. plantarum,* many bacteria including streptococci possess a manganese-cofactored superoxide dismutase A (SodA) which acts as a key enzyme in the removal of this toxic ROS. How does Mn get into the cell? This is controlled via the activity of an ABC cassette transporter known as PsaABC in *Streptococcus pneumoniae;* loss of this transporter leads to increased susceptibility to oxidative stress [3].

This type of transporter, whose binding protein is a member of the cluster IX family of Mn/Zn/Fe binding proteins, appears to be widespread in the bacterial world and it is found in a number of pathogens which seem to be able to grow under conditions of low iron. Such bacteria include *Treponema pallidum* and *Borrellia burgdorfii;* having a low iron requirement and combining the antioxidant chemistry of manganese with the use of a remarkable iron-sequestering protein known as Dps which can also bind to DNA to protect this macromolecule against damage by preventing iron-driven Fenton chemistry. This bio-inorganic chemistry may be a central survival mechanism for such bacteria in the host environment [4].

The most spectacular example of the use of manganese as an anti-oxidant is seen in *Deinococcus radiodurans* where accumulation of Mn is central to gamma-radiation resistance [5]. Measurement of intracellular Mn/Fe ratios showed that *D. radiodurans* has a ratio of Mn/Fe which is close to unity, about 50 times greater than that seen in *Escherichia coli.*

In addition to the primary manganese transporters described above, *Salmonella enterica* serovar Typhimurium and *E. coli* have been shown to possess a secondary transporter with sequence homology to

the Nramp 1 transporter of macrophages. The protein from *S. typhimurium* has been demonstrated to act as a Mn/proton symport and has been name MntH. An ABC cassette system known as *sitABC* in *S. typhimurium* is also present [6].

Manganese and bacterial pathogenicity

The importance of Mn transporters in bacterial pathogenicity is highlighted by recent studies of the virulence of *S. pneumoniae.* It had been known for some time that PsaA was an important virulence factor in *S. pneumoniae* since mutants lacking *psaA* were avirulent in a mouse-model system and immunisation with PsaA provided protective immunity against streptococcal infection. The reason for the attenuation of the *psa* mutants became clear when it was established that they were hypersensitive to oxidative stress arising from the loss of the Mn transporter [3].

Although there is no model system for testing the pathogenicity of *Neisseria gonorrhoeae,* we have shown that the MntABC Mn transport system is critical for defence against oxidative stress [7] and is required for survival of this bacterium in human cervical epithelial cells [Edwards, McEwan, Jennings, Apicella and co-workers, unpublished observations]. In *S. enterica* Typhimurium, it has been shown that mutation of *mntH* and *sitABC* attenuates virulence [8]. Thus, manganese uptake may of importance in the survival strategies of some mucosal and intracellular pathogens.

Regulation of Mn transport – MntR

A considerable amount of fundamental research on Mn metabolism has been carried out in *Bacillus subtilis,* where it has been known for many years that Mn is a requirement for sporulation [4]. This bacterium possesses both an ABC cassette system for Mn transport, MntABC and the secondary transporter MntH.

In *B. subtilis* it was shown that expression of these transporters was under the control of the transcription factor MntR. MntR is a paralogue of DtxR and appears to be found in all bacteria that possess a MntABC-like transporter for Mn (with the notable exception of *N. gonorrhoeae).* DtxR proteins are recognised as being repressors of gene expression in response to iron in Gram-positive bacteria. Proteins of the MntR branch of the DtxR superfamily act repressors in response to Mn and a crystal structure of MntR from *B. subtilis* has been reported which reveals two Mn ions coordinated [9].

In *B. subtilis* it has been shown that MntR differentially regulates the two major transporters; *mntH* expression is repressed when high intracellular Mn concentrations are reached while *mntABC* is repressed at lower Mn concentrations [10]. These data are consistent with MntABC having a key role in uptake of Mn when this divalent cation is limiting.

Manganese and global regulation of gene expression in Bacillus – the PerR regulon

More than a decade of research into the peroxide defence response in *E. coli* has led to a detailed model of global regulation of gene expression in Gram-negative bacteria controlled by the OxyR transcription factor. OxyR acts mainly as an activator of gene expression in response to peroxide stress and senses peroxide stress using redox-active cysteines.

There is no OxyR in *B. subtilis* and, instead, it appears that in this Gram-positive bacterium control of gene expression associated with peroxide stress is linked to the action of the transcription factor PerR which acts as a global repressor of genes encoding enzymes involved in the peroxide defence response including catalase and the Dps-like DNA binding protein MrgA [11]. PerR is a paralogue of Fur (the iron uptake regulator) and acts a repressor in a dimeric form. In standard media, PerR contains a structural Zn atom and a Fe ion and, under these conditions, the PerR regulon is rapidly induced upon exposure to low amounts of peroxide. Interestingly, under conditions where the Mn:Fe ratio is raised (iron limitation), manganese replaces iron in the PerR regulator and the result is a form of repressor that is relatively insensitive to hydrogen peroxide.

An outstanding recent study by Helmann's group [12] has shown that PerR does not use redox-active cysteines to sense peroxide. Instead, it was shown that, in the Fe form of PerR, iron and peroxide catalyse the formation of a hydroxyl radical which results in the formation of a 2-oxo histidine residue. This oxidised form of PerR cannot act as a repressor. Thus, the Fe:Mn ratio critically affects the sensitivity of PerR to peroxide and allows *B. subtilis* to grade its response to peroxide stress according to the level of iron in the cell.

Manganese and global gene expression: future directions

Clearly, manganese biochemistry has emerged as an area of major significance but its importance in the global regulation of gene expression is still far from understood. The use of PerR in *B. subtilis* represents a situation where Mn plays a role in the transcription of a regulon. Recently, we showed that a Fur-paralogue in *N. gonorrhoeae,* also named PerR, controls expression of *mntABC* and a small regulon that may be involved in manganese/zinc homeostasis [13].

It seems likely that more Mn-dependent transcriptional regulators will emerge but an equally important area of research will be to determine how manganese exerts its effects at the post-transcriptional and post-translational level. There are a large number of Mn-dependent enzymes, many of which are involved in central carbon metabolism; in their extensive analysis that focused on *E. coli* and *S. enterica* Typhimurium, Kehres & Maguire [6] suggested the Mn may modulate enzyme networks with the regulation of intermediary carbon metabolism being a potentially important target. These authors have also proposed that increasing intracellular Mn concentrations, that appear to accompany a slow down in growth or stasis, may have a key role in regulating enzyme activities that affect bacterial persistence.

Our knowledge of this area is still cursory, but studies of the role of Mn in global changes of gene expression at the

transcriptome and proteome levels should be a fruitful area of research for those interested in general bacterial physiology and in bacterial pathogenicity.

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NATA Forensic Module Accreditation is applicable to microbiology laboratories

NATA has launched a new forensic science accreditation programme, the Forensic Operations module [1].

NATA report that this programme is quite a change to the way traditional NATA programmes operate. It has been developed specifically for laboratories currently accredited in other fields of testing (as distinct from forensic science) that are involved or interested in testing for legal purposes.

The experience of MDU PHL as a microbiology laboratory can be found on p9 of the same *NATA News* issue. In short, the accreditation experience generally enhanced the quality systems of the laboratory as it relies and builds on the existing technical accreditation. MDU PHL would be delighted to share their experience with anyone contemplating forensic accreditation via this new route.

1. NATA News – 20 March 2006
http://www.nata.asn.au/index.cfm?objectId=F6EBCB13-D4DD-7F53-95515F452BDBDB54

Contact: Geoff Hogg FRCPA FRACP BMBS BVSc BMedSci MVS JD GDLP
Director, Microbiological Diagnostic Unit, Public Health Laboratory
Microbiology & Immunology Department
The University of Melbourne, VIC 3010
Tel: (03) 8344 5701
Fax: (03) 8344 7833
Pager: (03) 9625 2689
E-mail: g.hogg@mdu.unimelb.edu.au

Non RCPA/NATA accredited (biological type) testing in medical laboratories

Medically accredited laboratories are often asked to perform tests outside the usual classes found in such accreditation. Some of these tests are common and familiar to medical labs, such as those relating to implanted and removed foreign bodies. Others, such as testing of food and water, clearly come under NATA biological accreditation

In between there is a grey zone where the organisms being sought are familiar to medical testing labs but the testing is not directly related to the patient; these tests would currently fall under biological accreditation. Examples include testing endoscope washings (for those who choose to do so) and environmental infection control investigations.

There are many other problematic areas. Recognising the importance of a medical lab being able to have all testing they conduct accredited for quality purposes (and not just HIC) and to empower labs to refer tests for which they are not accredited or experienced, RCPA and NATA are working together toward a solution within the next year. The RCPA effort is being led by Colin Macleod who is happy to receive comments. Views for NATA could be channelled through NATA elected reps.

Contact: Colin Macleod
E-mail: colin.macleod@email.cs.nsw.gov.au
Web: http://www.nata.asn.au/index.cfm?objectid=1D52DAB7-A8E8-1AC3-6AB2CA0ABFD776E1