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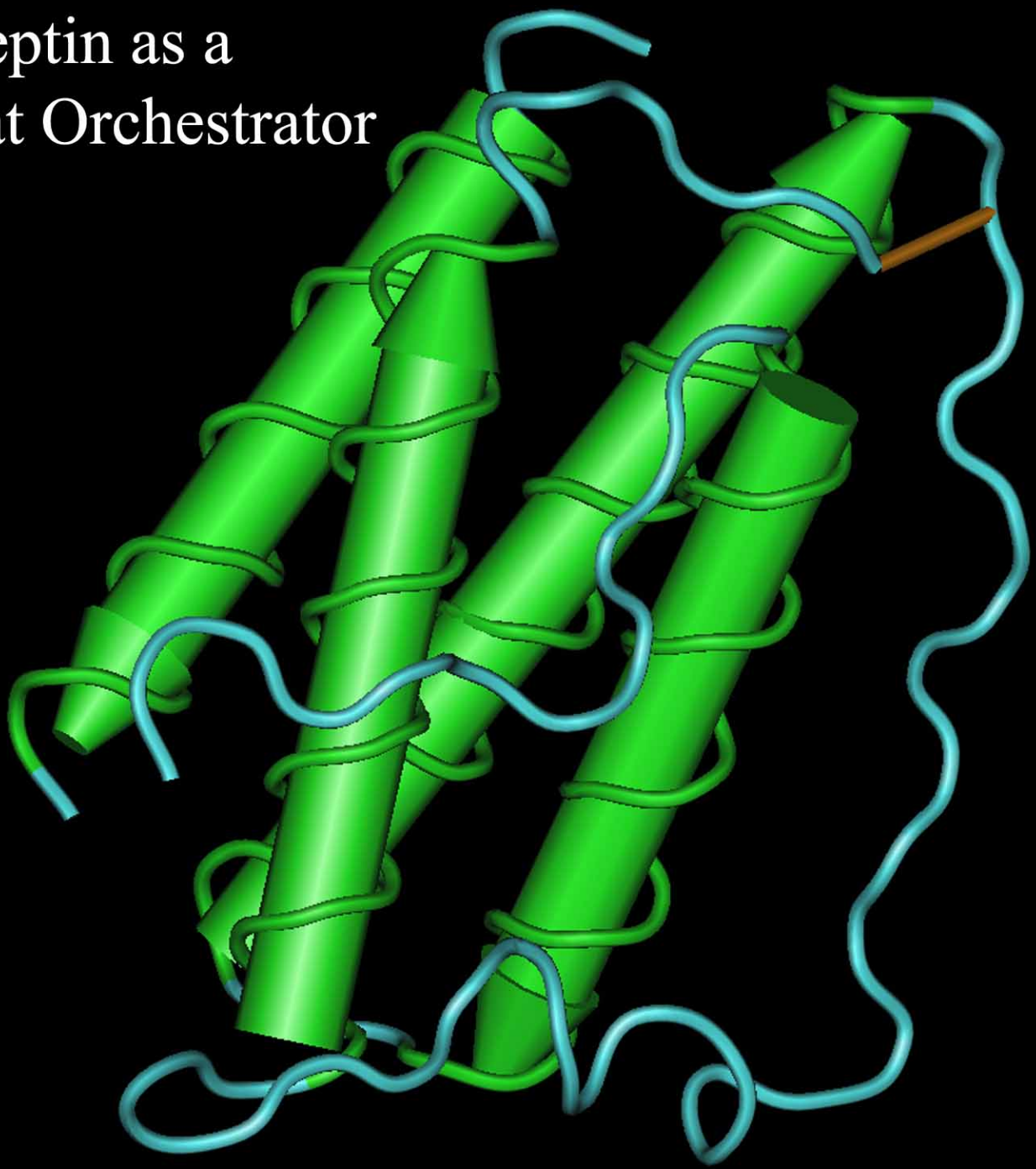
Leptin as a Fat Orchestrator



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Annual RUR

Letter from the Editor & Staff

Dear Reader,

We began this journal two years ago with the goal of providing a forum through which undergraduates interested in research could present their work at a professional level, giving them the exposure and experience to help them transition into such a career.

Last year, we had an outstanding inaugural issue. We published five articles covering a wide range of topics written by students from across the United States. This year, we were happy to have expanded our journal to an international level. Inside our second annual issue, you will find four articles, again covering a variety of topics from molecular dynamics to molecular biology.

Once again, we would like to thank Rice University for sponsoring us and all of the people who have contributed to helping us meet our goals with RUR.

Akash J. Patel
Editor-in-Chief, RUR
Rice University, '03

Questions or Comments regarding RUR and those interested in submitting a review for the 2004 issue should direct their inquiries to the 2004 Editor-in-Chief, Peter Lee at “pllee@rice.edu”.

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About RUR:
Reviews in Undergraduate Research is an international undergraduate research publication put together by the students of Rice University in Houston, TX. RUR aims to provide an international forum where undergraduate students can share knowledge of current natural sciences research with their peers around the world.

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Implications of Microbial Heavy Metal Tolerance in the Environment

Anne Spain[1], Communicated by: Dr. Elizabeth Alm[2]

Although some heavy metals are essential trace elements, most can be, at high concentrations, toxic to all branches of life, including microbes, by forming complex compounds within the cell. Because heavy metals are increasingly found in microbial habitats due to natural and industrial processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals (by either efflux, complexation, or reduction of metal ions) or to use them as terminal electron acceptors in anaerobic respiration. Thus far, tolerance mechanisms for metals such as copper, zinc, arsenic, chromium, cadmium, and nickel have been identified and described in detail. Most mechanisms studied involve the efflux of metal ions outside the cell, and genes for this general type of mechanism have been found on both chromosomes and plasmids. Because the intake and subsequent efflux of heavy metal ions by microbes usually includes a redox reaction involving the metal (that some bacteria can even use for energy and growth), bacteria that are resistant to and grow on metals also play an important role in the biogeochemical cycling of those metal ions. This is an important implication of microbial heavy metal tolerance because the oxidation state of a heavy metal relates to the solubility and toxicity of the metal itself. When looking at the microbial communities of metal-contaminated environments, it has been found that among the bacteria present, there is more potential for unique forms of respiration. Also, since the oxidation state of a metal ion may determine its solubility, many scientists have been trying to use microbes that are able to oxidize or reduce heavy metals in order to remediate metal-contaminated sites.

Another implication of heavy metal tolerance in the environment is that it may contribute to the maintenance of antibiotic resistance genes by increasing the selective pressure of the environment. Many have speculated and have even shown that a correlation exists between metal tolerance and antibiotic resistance in bacteria because of the likelihood that resistance genes to both (antibiotics and heavy metals) may be located closely together on the same plasmid in bacteria and are thus more likely to be transferred together in the environment. Because of the prevalence of antibiotic resistant pathogenic bacteria, infectious diseases are becoming more difficult and more expensive to treat; thus we need to not only be more careful of the drastic overuse of antibiotics in our society, but also more aware of other antimicrobials, such as heavy metals, that we put into the environment.

INRODUCTION

This paper focuses on the general ways in which microbes interact with metals. Some bacteria have evolved mechanisms to detoxify heavy metals, and some even use them for respiration. Microbial interactions with metals may have several implications for the environment. Microbes may play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up or remediating metal-contaminated environments. There is also evidence of a correlation between tolerance to heavy metals and antibiotic resistance, a global problem currently threatening the treatment of infections in plants, animals, and humans.

Metal Tolerance Mechanisms

In high concentrations, heavy metal ions react to form toxic compounds in cells (Nies, 1999). To have a toxic effect, however, heavy metal ions must first enter the cell. Because some heavy metals are necessary for enzymatic functions and bacterial growth, uptake mechanisms exist that allow for the entrance of metal ions into the cell. There are two general uptake systems — one is quick and unspecific, driven by a chemiosmotic gradient across the cell membrane and thus requiring no ATP, and the other is slower and more substrate-specific, driven by energy from ATP hydrolysis. While the first mechanism is more energy efficient, it results in an influx of a wider variety of heavy metals, and when these metals are present in high concentrations, they are more likely to have toxic effects once inside the cell (Nies and Silver, 1995).

To survive under metal-stressed conditions, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell, and reduction of the heavy metal ions to a less toxic state (Nies, 1999). Mergeay et al. (1985) tested the minimal inhibitory concentrations (MICs) of several different metal ions for *Escherichia coli* on agar medium, and the most toxic metal (with the lowest MIC) was mercury, whereas the least toxic metal tested was manganese (Table 1). Three examples of metal ions to which bacteria have evolved well-studied resistance mecha-

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Heavy Metal	MIC (mM)
Mercury	0.01
Silver	0.02
Gold	0.02
Chromium (Cr(VI))	0.2
Palladium	0.2
Platinum	0.5
Cadmium	0.5
Cobalt	1
Nickel	1
Copper	1
Zinc	1
Thallium	2
Uranium	2
Lanthanum	2
Yttrium	2
Scandium	2
Ruthenium	2
Aluminum	2
Lead	5
Iridium	5
Osmium	5
Antimony	5
Indium	5
Rhodium	5
Gallium	5
Chromium (Cr(II))	5
Vanadium	5
Titanium	5
Beryllium	5
Chromium (Cr(III))	10
Manganese	20

Table 1. Minimal inhibitory concentrations (MICs) of several heavy metals in *Escherichia coli*. The MICs were determined on an agar medium at different acidities, allowing for the dissolution of the metal ions. Minimal inhibitory concentrations refer to the smallest concentration necessary to inhibit growth; thus, lower MIC values indicate more toxic metals and higher MICs indicate less toxicity.

nisms – copper, zinc, and arsenic – are illustrated in this review.

Copper

Copper is used by cells in small quantities in cellular enzymes (e.g., cytochrome c oxidase). However, because copper is so widely used in mining, industry, and agriculture, high levels of copper may exist in some environments. As such, bacteria have evolved several types of mechanisms to resist toxicity due to high copper concentrations. With respect to the prevalence of copper resistance in the environment, Lin and Olson (1995) studied bacteria isolated from a water distribution system experiencing copper corrosion, and 62% were found to be copper resistant. Of these resistant bacteria, 49% had *cop* or *cop*-like gene systems, including both compartmentalization and efflux systems (Cooksey, 1993).

In the plant pathogen *Pseudomonas syringae*, resistance to copper via accumulation and compartmentalization in the cell's periplasm and outer membrane is due to four proteins encoded on the plasmid-borne *cop* operon (Cooksey, 1994). The proteins are found in the periplasm (CopA and CopC), the outer membrane (CopB), and the inner membrane (CopD) and work together to compartmentalize copper away from sensitive cellular functions. In *E. coli*, resistance to copper is based on an efflux mechanism by which copper is removed from the cell. The efflux proteins are expressed by plasmid-bound *pco* genes, which are in turn are dependent on the expression of chromosomal *cut* genes (Cooksey, 1993). Two *cut* genes (*cutC* and *cutF*) were identified by Gupta et al. (1995) and were shown to encode a copper-binding protein and an outer membrane lipoprotein. Cooksey (1993) also states that most bacterial species in the environment have acquired at least one of the aforementioned copper management systems, and that the evolution of copper resistance may have come about through the modification of copper uptake genes found on chromosomes.

Zinc

Zinc is another essential trace element. It is not biologically redox reactive and is thus not used in respiration. It is, however, important in forming complexes (such as zinc fingers in DNA) and as a component in cellular enzymes (Nies, 1999). Bacterial cells accumulate zinc by a fast, unspecific uptake mechanism and it is normally found in higher concentrations (but is less toxic) than other heavy metals (Nies, 1999). Uptake of zinc ions is generally coupled to that of magnesium, and the two ions may be transported by similar mechanisms in bacteria (Nies and Silver, 1995).

Two general efflux mechanisms are responsible for bacterial resistance to zinc. One is a P-type ATPase efflux[1] system that transports zinc ions across the cytoplasmic membrane by energy from ATP hydrolysis. A chromosomal gene, *zntA*, was isolated from *E. coli* K-12 and was found to be responsible for the ATPase that transports zinc and other cations across cell membranes (Beard et al., 1997). The other mechanism involved in zinc efflux is an RND-driven[2] transporter system that transports zinc across the cell wall (not just the membrane) of gram-negative bacteria and is powered by a proton gradient and not ATP (Nies, 1999).

[1] A P-type ATPase is defined as an ATPase that forms a phosphorylated intermediate while catalyzing a reaction (Nies and Silver, 1995).

[2] RND refers to a family of proteins that are involved in the transport of heavy metals (Nies, 1999).

Arsenic

Arsenic, which is not considered a heavy metal but rather a semi-metal with metallic and non-metallic properties, is toxic to bacteria, as well as other domains of life. For arsenic to have toxic effects, though, it first needs to be in a bioavailable form. Arsenic uptake by bacteria is mediated by phosphate transporters and is generally pumped back out of the cell by an efflux pump (Nies and Silver, 1995).

Several mechanisms for resistance to arsenic have been identified. Chen et al. (1986) proposed a model for the plasmid-mediated mechanism of the efflux of arsenate and arsenite in gram-negative bacteria. The nucleotide sequence of a fragment of DNA containing the *ars* operon[3] was studied, and three genes, *ars*A, *ars*B, and *ars*C, were found to encode for the proteins ArsA, ArsB, and ArsC, respectively. ArsA is a protein with ATPase activity and thus is involved in translocation of the metal ions across the cell membrane. ArsB interacts with ArsA on the inner membrane of the cell, and the two proteins form the arsenite pump. ArsC is a smaller protein that alters the specificity of the arsenite pump to allow for the efflux of arsenate. Thus, ArsC is only required for tolerance to arsenate, and ArsA and ArsB are required for tolerance to both species of arsenic.

Gladysheva et al. (1994) isolated and studied the protein ArsC encoded by the *arsC* gene on plasmid R773 and found that the protein actually catalyzes the reduction of arsenate to arsenite in *E. coli*, using NADPH as the reducing power; this suggests that the arsenite pump is not altered by the ArsC protein, but that it is rather the substrate (arsenate) is altered (or reduced) to fit the arsenite pump. Ji et al. (1994) isolated and purified another arsenate reductase protein; this one, however, was encoded by a gene on plasmid p1258 of *Staphylococcus aureus*, a gram-positive bacterium. This arsenate reductase (ArsR) was found to be active in the presence of thioredoxin and NADPH.

A chromosomal operon homologous to the *ars* operon found on plasmid R773 was identified in *E. coli* (Dioro et al., 1995), and was also responsible for encoding resistance to arsenic. Dioro suggests that this chromosomal operon might have been a precursor to plasmid-mediated arsenic resistance mechanisms involving the reduction of arsenate.

Arsenic Biogeochemistry – An Example of Microbial Interactions with Metals in the Environment

Because arsenic is also toxic to humans and is a known carcinogen, the United States Environmental Protection Agency (US EPA) has established a maximum contaminant concentration level of 50 μg/L of arsenic in drinking water, which is proposed to go down to 10 μg/L within several years (http://www.epa.gov/ogwdw000/ars/ars9.html, visited 1/08/01). Despite these mandates, however, arsenic contamination remains a worldwide threat. Arsenic concentrations are higher in groundwater than in surface water where the presence of arsenic is mainly due to dissolved minerals from weathered rocks and soils. The United States Geological Survey (USGS) found that 10% or more of groundwater in several counties in the Midwest and Northeast U.S. exceeded arsenic concentrations of 50 μg/L (http://co.water.usgs.gov/tace/arsenic, visited 1/08/01). Additionally, in groundwater from the area surrounding and including Hanoi, Vietnam, arsenic concentrations have been found to range from 1-3050 μg/L with an average concentration of 159 μg/L. In highly affected areas, arsenic concentrations averaged over 400 μg/L. Water analyzed after treatment processes had concentrations ranging from 25-91μg/L, but with 50% of wells tested still being over the 50 μg/L arsenic concentration standard (Berg et al., 2001). High arsenic concentrations pose a significant chronic health threat to millions drinking contaminated water, and in some groundwater, concentrations of arsenic are indeed high enough to allow for arsenic resistance mechanisms in microbes to remain ecologically favorable.

Many studies have been done on microbial metabolism of arsenic in aquatic environments and the effects microbes have on the speciation and mobilization of arsenic. Since aquatic sediments can be anaerobic, and because arsenic concentrations in sediments can range from 100-300 μg/L, microbe-mediated arsenic reduction may be common. Brannon and Patrick (1987) found that the addition of arsenate to an anaerobic sediment resulted in the accumulation of arsenite, indicating the reduction of arsenate to arsenite by microbes. Ahmann et al. (1997) further showed that native microorganisms from the Aberjona watershed were, in fact, responsible for the arsenic flux in the anoxic contaminated sediments. Harrington et al. (1998) also demonstrated the ability of microbes in sediments from Coeur d'Alene Lake to reduce arsenate. In reducing conditions, it was found that arsenite was the dominant form of arsenic. They also found that dissimilatory iron-reducing bacteria (DIRB) and sulfate-reducing bacteria (SRB) are capable of both arsenic reduction and oxidation and thus may contribute to the cycling of arsenic in sediments.

Microbial reduction of arsenate in aquatic sediments is important because arsenite (the reduced form) is more toxic and more soluble (and thus, more mobile) than arsenate, which forms relatively insoluble, non-bioavailable compounds with ferrous oxides and manganese oxides. Speciation of arsenic is affected or controlled by not only oxidation and reduction processes by microbes, but also by methylation by microbes, and adsorption to other particles (Aurilio et al., 1994). It was found that DIRB responsible for the dissolution of iron oxides bound to arsenic can also free soluble arsenic into the sediment (Cummings et al., 1999). Another study done on arsenic biogeochemistry in Lake Biwa Japan showed that arsenic concentration and speciation may also depend on eutrophication[4] (Sohrin et al, 1997).

[3] *ars* operon refers to the operon found on plasmid R773 in gram-negative bacteria that encodes for the efflux of arsenate and arsenite

Many scientists have sought microbial community members responsible for arsenate reduction. Hoeft et al. (2002) found that, in the anoxic water of Mono Lake (California), two subgroups (Sulfurospirillium and Desulfovibrio) of the Proteobacteria lineage were present and most likely using arsenate as an electron acceptor for growth. They also found an interesting cycling of arsenic occurring; the presence of nitrate rapidly re-oxidized any arsenate that had been produced. Thus, in some environments, both oxidation and reduction of arsenic may occur. In another study of aerobic contaminated mine tailings, it was found that members of the *Caulobacter*, *Sphingomonas*, and *Rhizobium* families may be responsible for the reduction and mobilization of arsenic (Macur et al., 2001).

While it has been shown that microbes are capable of arsenic reduction, the question remains whether microbes reduce arsenate for detoxification purposes (as described in the section on arsenic tolerance mechanisms) or for growth during anaerobic respiration. In a sample of agricultural soil, it was determined that the reduction of arsenate was not involved in respiration because rates of arsenate reduction did not contribute to microbial growth (Jones et al., 2000). Thus, arsenate reduction in this case is probably due to intracellular detoxification by mechanisms, similar to those described in *E. coli* and *S. aureus*. Conversely, Laverman et al. (1995) showed that the bacterial strain SES-3 could grow using a diversity of electron acceptors, including Fe(III), thiosulfate, and arsenate coupled to the oxidation of lactate to acetate. Another study reported the growth of strain MIT-13 (isolated from the Aberjona watershed) by using arsenate as an electron acceptor, and the inhibition of arsenate reduction by molybdate (Ahmann et al., 1994). Additionally, an organism from the genus *Desulfitobacterium* isolated from lake Coeur d'Alene was shown to reduce arsenate, but it was not determined whether this reduction supported growth (Niggemeyer et al., 2001). From arsenic-contaminated mud from Australia, however, a *Bacillus* strain was isolated and characterized as being able to respire with arsenate (Santini et al., 2002).

Uranium Reduction – An Example of Microbial Metal Bioremediation

Because of radionuclides present in soils and groundwater due to nuclear waste during the Cold War Era, much effort has been put forth to see whether microbes can contribute to remediation by reducing and immobilizing toxic metals, such as uranium (Francis and Dodge, 1998). It has been shown that DIRB in the family *Geobacteraceae* are involved in uranium reduction in contaminated aquifer sediments (Holmes et al., 2002) and also in technetium reduction (Lloyd et al., 2000). Certain sulfate reducers have also been shown to be capable of reducing uranium; in *Desulfovibrio vulgaris*, it was shown that cytochrome c_3 was responsible and necessary for uranium reduction activity (Lovley et al., 1993). Additionally, members of *Clostridium* species have been shown to reduce uranium under anaerobic conditions (Francis et al., 1994). This has implications in the bioremediation of uranium contaminated aquifers and sediments, as soluble and mobile uranium poses much more of a threat to public health and the environment than an insoluble precipitate.

Correlation of Metal Tolerance and Antibiotic Resistance

Bacterial resistance to antibiotics and other antimicrobial agents is an increasing problem in today's society. Resistance to antibiotics is acquired by a change in the genetic makeup of a bacterium, which can occur by either a genetic mutation or by transfer of antibiotic resistance genes between bacteria in the environment (American Academy of Microbiology, 2000).

Because our current antibiotic are becoming less useful but used more heavily against antibiotic resistant pathogenic bacteria, infectious diseases are becoming more difficult and more expensive to treat. The increased use of antibiotics in health care, as well as in agriculture and animal husbandry, is in turn contributing to the growing problem of antibiotic-resistant bacteria. Products such as disinfectants, sterilants, and heavy metals used in industry and in household products are, along with antibiotics, creating a selective pressure in the environment that leads to the mutations in microorganisms that will allow them better to survive and multiply (Baquero et al., 1998).

According to Jeffrey J. Lawrence's (2000) discussion of the Selfish Operon Theory, clustering of genes on a plasmid, if both or all genes clustered are useful to the organism, is beneficial to the survival of that organism and its species because those genes are more likely to be transferred together in the event of conjugation. Thus, in an environment with multiple stresses, for example antibiotics and heavy metals, it would be more ecologically favorable, in terms of survival, for a bacterium to acquire resistance to both stresses. If the resistance is plasmid mediated, those bacteria with clustered resistance genes are more likely to simultaneously pass on those genes to other bacteria, and those bacteria would then have a better chance at survival. In such a situation, one may suggest an association with antibiotic resistance and metal tolerance. For example, Calomiris et al. (1984) studied bacteria isolated from drinking water and found that a high percent of bacteria that were tolerant to metals were also antibiotic resistant.

CONCLUSIONS

Although some heavy metals are important and essential trace elements, at high concentrations, such as those found in many environments today, most can be toxic to microbes. Microbes have adapted to tolerate the presence of metals or can even use them to grow. Thus, a number of interactions

[4] Eutrophication is a condition in which the presence of large amounts of biomass/organic matter in surface waters due to high nutrient loads results in low oxygen concentrations, poor water quality, and fish kills.

between microbes and metals have important environmental and health implications. Some implications are useful, such as the use of bacteria to clean up metal-contaminated sites. Other implications are not as beneficial, as the presence of metal tolerance mechanisms may contribute to the increase in antibiotic resistance. Overall, it is most important to remember that what we put into the environment can have many effects, not just on humans, but also on the environment and on the microbial community on which all other life depends.

ABOUT THE AUTHOR

Anne Spain carried out research related to the material in this article at Central Michigan University in Mt. Pleasant, Michigan from the fall of 2000 until the spring of 2002. Her work focused on finding a correlation between heavy metal tolerance and antibiotic resistance in *Escherichia coli* that had been isolated from central Michigan recreational waters. She also worked on trying to characterize the microbial community of arsenic-contaminated aquifers from several counties in Southeastern Michigan by amplification of community 16S rDNA and analysis by polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis. Due to complications with PCR, however, and limited time at the university, the project was not completed. Anne is currently enrolled as a graduate student at the University of Oklahoma where she is studying environmental microbiology and microbial ecology. She hopes to obtain her PhD after several years and continue in academia, conducting research and eventually teaching.

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The Role of Macrophages in Apoptosis: Initiator, Regulator, Scavenger

ZhaoYing Pearline Teo[1], Communicated by: Dr. David Hughes[2]

Macrophages play an integral role in the immune system. They are professional phagocytes, responsible for the recognition and engulfment of pathogens and toxins. Usually, phagocytosis of pathogens leads to macrophage activation, inducing the release of pro-inflammatory cytokines such as IL1, IL6 and TNF, as well as other toxic mediators that cause non-specific tissue damage. In addition to their immune functions, macrophages also participate in the clearing of apoptotic cells. Apoptosis is crucial in the development and homeostasis of all multicellular organisms. Typical characteristics of apoptotic cells include nuclear fragmentation, membrane blebbing and presentation of 'eat-me' signals. Membrane integrity is preserved, and cells are neatly engulfed by neighbouring cells and professional phagocytes. In contrast to the phagocytosis of pathogens, apoptotic clearing does not lead to inflammation. In fact, anti-inflammatory cytokines such as transforming growth factor â1 and prostaglandin E_2 are produced instead. This paper reviews the known macrophage receptors that mediate apoptotic clearing. It also looks at recent evidence that implicate phagocytes in the induction and regulation of programmed cell death.

Macrophages and apoptotic clearance

Macrophages express a variety of receptors for pathogen associated molecular pathways (PAMPs) such as lipopolysaccharide (LPS) and mannose receptors. These enable the recognition and phagocytosis of a large repertoire of pathogens, and the subsequent generation of inflammation to eradicate the infection. Interestingly, many of the same receptors are also involved in the recognition and clearing of self-apoptotic cells. Yet, instead of macrophage activation and production of pro-inflammatory cytokines, anti-inflammatory mediators are released, and clearing is quickly and painlessly performed. However, mouse peritoneal macrophages ingesting apoptotic T cells have been observed to secrete the proinflammatory chemokine MIP-2, suggesting that some degree of macrophage activation may occur under certain circumstances (Uchimura et al., 1997).

If the rate of apoptosis exceeds the clearing capacity of macrophages, apoptotic cells may become necrotic, resulting in the release of harmful cellular contents and damage to surrounding tissue (fig 1). Treatment of mice with anti-Fas antibody triggered a massive wave of apoptosis in the liver, leading to extensive hepatic necrosis and death (Ogasawara et al., 1993). Hence, clearance is a crucial step in the resolution of apoptosis and containment of cell death.

The exact interactions between apoptotic cells and macrophages have not been fully elucidated, but it appears to involve multiple, partially redundant pathways. Apart from the well-known phosphatidylserine (PS) 'eat-me' signal (Fadok et al., 1992; Fadok et al., 2001; Schlegel and Williamson, 2001), other surface markers of apoptotic cells have not been well characterised. On the other hand, macrophage receptors are better known, and several promising candidates have been identified, including scavenger receptors, CD36, CD14 and the PS receptor (fig 2).

Class A Scavenger Receptors

The scavenger receptors (SRs) are a structurally diverse family of receptors with broad ligand specificities. SR-A binds acetylated and oxidised low-density lipoprotein (LDL), and polyanionic compounds such as maleylated bovine serum albumin and polyinosinic acid (Pearson, 1996). The addition of monoclonal antibodies to SR-A, or polyanionic ligands significantly inhibits the in vitro phagocytosis of apoptotic thymocytes by thymus-derived macrophages (Platt, 1996). Further studies with thymic macrophages from SR-A null mice show that although phagocytosis of thymocytes was inhibited by 50%, the relative number of apoptotic thymocytes in these mice was not appreciably larger. This suggests that other receptors are sufficient for normal apoptotic clearance in the thymus (Platt, 1998).

Class B Scavenger Receptors and the Vitronectin Receptor

The class B scavenger receptor CD36 recognises a range of ligands, including typeI collagen, thrombospondin, oxidised LDL and PS (Rigotti, 1995). It is observed that the plasma protein thrombospondin 1 (TSP1) acts to bridge apoptotic cells, CD36 (a TSP1 receptor) and the vitronectin receptor ($á_v â_3$ integrin) (Savill et al., 1990; Savill et al., 1992; Stern et al., 1996; Akbar, 1994). CD36 gene transfer to amateur phagocytes such as melanoma cells boosts their capacity for uptake of apoptotic neutrophils to near-profes-

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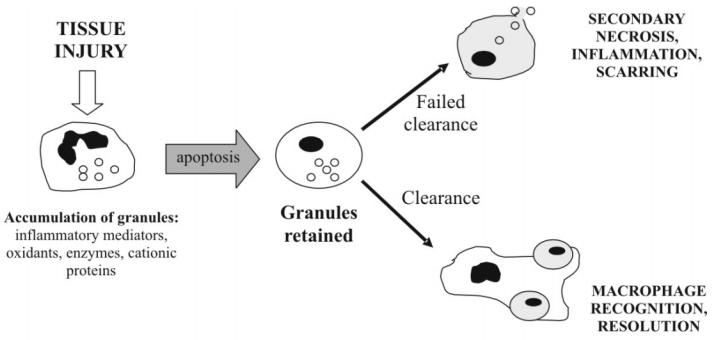

Fig 1. Neutrophil apoptosis and phagocytic clearance by macrophages in the resolution of inflammation

sional levels. Antibodies to $\alpha_v\beta_3$ and TSP1 inhibit this improvement (Ren et al., 1995). Conflicting evidence exists for the importance of CD36 in phagocytizing apoptotic cells in vivo. On one hand, blood monocytes from SLE patients demonstrate a decrease in CD36 levels paralleled by a deficiency in the phagocytosis of apoptotic cells. On the other hand, monocyte-derived macrophages from CD36-deficient patients show no defect in the phagocytosis of apoptotic neutrophils (Platt, 1998). This reflects the considerable redundancy that underlies the uptake of apoptotic cells.

CD14

A monoclonal antibody that specifically inhibited internalisation of intact ageing neutrophils (PMNs) by monocyte-derived macrophages was found to recognise CD14, a molecule also known to recognise LPS. Although apoptotic cells bind to CD14 at a site close to the LPS-binding site, they do not induce the severe inflammation produced by LPS (Devitt, 1998). LPS normally binds CD14 in the presence of serum LPS binding protein (LBP). The complex then interacts with macrophage toll-like receptor 2 (TLR2) to result in macrophage activation (Yang, 1998). It is possible that CD14 does not interact with TLR2 during apoptotic clearing due to the absence of LBP, thus triggering a different response.

PS Receptors

It is widely accepted that phosphatidylserine (PS) exposure on the outer leaflet of the plasma membrane is an important signal displayed by preapoptotic cells (Fadok et al., 1992; Fadok et al., 2001; Schlegel and Williamson, 2001). Macrophage phagocytosis of apoptotic lymphocytes was inhibited, in a dose-dependent manner, by liposomes containing phosphatidyl- L-serine, but not by liposomes containing other anionic phospholipids, including phosphatidyl- D-serine (Fadok et al., 1992). Yet, the nature and identity of the macrophage PS receptor had remained a mystery. A recent publication by Fadok et al reports the cloning of a candidate receptor, identified using monoclonal antibodies against human macrophages. Transfection of the gene into Jurkat T cells (which are negative for the receptor) confers the ability to bind and engulf apoptotic cells. PS liposomes, but not phophatidylinositol (PI) nor phosphatidylcholine (PC) liposomes, inhibit the binding, suggesting that the cloned protein is a PS-specific receptor involved in the recognition and engulfment of apoptotic cells (Fadok et al., 2000). Furthermore, stimulation of this receptor on different types of phagocytes by apoptotic cells, PS- containing liposomes or an IgM monoclonal anti-PS antibody initiates release of TGF-â, known to be involved in the anti-inflammatory effects of apoptotic cells (Fadok et al., 2001).

Another protein implicated in PS recognition is milk fat globule-EGF-factor 8 (MFG-E8) (Hanayama, 2002). A purified recombinant form, MFG-E8-L, binds to thymocytes treated with dexamethasone, which induces apoptosis. This interaction is reduced by pre-treatment of the cells by Annexin V, which binds PS. MFG-E8-L was found to bind to PS and PE, but not PC and PI. It also contains an arginine-glycine-aspartate (RGD) motif, which can be recognised by integrins. MFG-E8-L enhances phagocytosis of apoptotic thymocytes by mouse NIH3T3 cells, especially those NIH3T3 cells transformed to express a high level of $\alpha_v\beta_3$ integrin. Integrins have been suggested as receptors for apoptotic cells in several systems (Savill et al., 1990; Albert et al., 1998). However, because neither $\alpha_v\beta_3$ or $\alpha_v\beta_5$ integrin can bind PS, it has not been clear how these integrins recognize apoptotic cells. MFG-E8-L seems to resolve this dilemma.

Multiple receptors appear to respond to PS exposure. Pradhan et al demonstrated that both activated and unactivated macrophages recognize PS, but with different receptor systems. Phagocytosis of apoptotic lymphocytes by activated (but not by unactivated) macrophages is inhibited by pure PS vesicles as well as by N- acetylglucosamine, implicating involvement of a lectin-like receptor in this case.

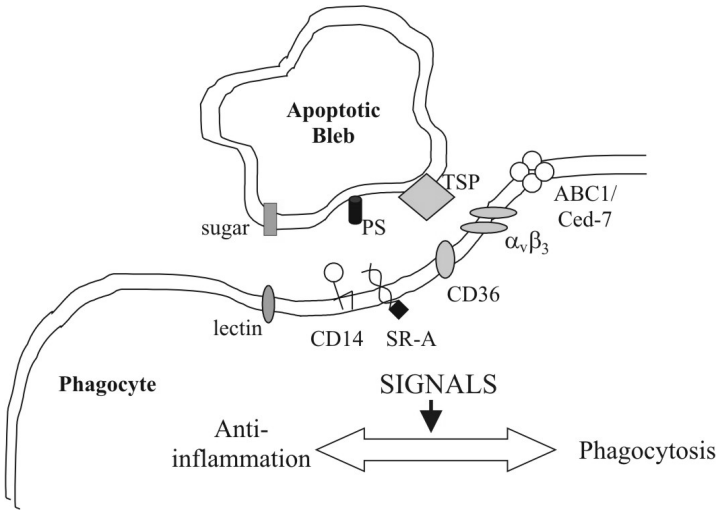

Fig. 2. Phagocytosis of an apoptotic cell by a macrophage. Recognition of the apoptotic cell is mediated by a variety of receptors including lectins, CD14, scavenger receptor A (SR-A), and CD36 in conjunction with the vitronectin receptor. Recognition signals on the apoptotic cell include sugars, phosphatidylserine (PS), and surface-bound thrombospondin (TSP).

Conversely, uptake of apoptotic lymphocytes by unactivated (but not by activated) macrophages is inhibited by PS on the surface of erythrocytes as well as by the tetrapeptide RGDS and cationic amino acids and sugars, implicating involvement of the vitronectin receptor in this case. Recognition by both classes of macrophages is blocked by the monocyte-specific monoclonal antibody 61D3. The signal recognized by activated macrophages appears to develop on the lymphocyte prior to assembly of the signal recognized by unactivated macrophages. Collectively, these results suggest that PS exposure on the surface of apoptotic lymphocytes generates a complex and evolving signal recognized by different receptor complexes on activated and unactivated macrophages (Pradhan, 1997).

Fadok et al proposes that the myriad of macrophage receptors may provide the strong adhesion needed to increase the likelihood of contact between the PS Receptor and its phospholipid ligand, which is required for uptake (Fadok et al., 2001). Interestingly, PS is exposed on both apoptotic and necrotic cells, and is not responsible for the differential macrophage responses invoked upon phagocytosis. Necrotic cells, when recognized, enhance proinflammatory responses of activated macrophages, although they are not sufficient to trigger macrophage activation. In marked contrast, apoptotic cells profoundly inhibit phlogistic macrophage responses; this represents a cell-associated, dominant-acting anti-inflammatory signaling activity acquired posttranslationally during the process of physiological cell death (Cocco and Ucker, 2001).

Genetic parallels in *C.elegans*

Although apoptotic clearance in *C. elegans* is performed by neighbouring, non-professional cells, genetic analyses has revealed two partially redundant engulfment pathways which might be applicable to macrophages. The two groups are *ced-1*, *ced-6* and *ced-7*, and *ced-2*, *ced-5*, *ced-10* and *ced-12* (Ellis et al., 1991). *ced-1*, *ced-6* and *ced-7* encode a scavenger receptor-like protein, a PTB-domain containing adaptor protein and an ABC transporter, respectively. They are components of a conserved signalling pathway in phagocytic cells, required for the recognition of an unknown cell-corpse signal (Hengartner, 2001). *ced-7* also functions in apoptotic cells, where it might be involved in the generation of this signal. *ced-2, ced-5, ced-10* and *ced-12* encode homologues of mammalian CrkII, Dock180, Rac and ELMO, which function to transduce another unknown cell-corpse signal to the actin cytoskeleton of the phagocytic cell (Conradt, 2001).

Phagocytes and the initiation of apoptosis

In most cases, blocking engulfment or eliminating phagocytes does not prevent the demise of doomed cells. However, some exceptions have been observed. For example, activated macrophages cocultured with myofibroblast-like mesangial cells in vitro can cause these cells to die (Duffield et al., 2000). In addition, macrophage elimination prevents capillary regression in the rat's eye in vivo, but reconstitution with unactivated macrophages restores the normal phenotype (Lang and Bishop, 1993; Diez-Roux and Lang, 1997). Finally, the death of the male-specific linker cell in *C. elegans* is prevented by inactivating one of the two engulfment pathways or by ablating the two neighbouring cells normally responsible for the engulfment of this cell. Thus, it appears that phagocytes are capable of inducing apoptosis. In mammalian systems, mediators of macrophage cytotoxicity in vitro include TNF-α (van de Loosdrecht, 1993) and nitric oxide (Cui, 1994).

Positive feedback cycle between phagocytes and apoptotic cells

Recent work in *C. elegans* has led to the discovery of a positive feedback loop between the engulfment machinery in phagocytic cells and the cell-death machinery in apoptotic cells. It was demonstrated that mutations that block engulfment strongly enhance the ability of partial lf (loss of function) mutations of pro-apoptotic genes (*ced-3* [caspase], *ced-4* [CED-3 activator], *egl-1* [CED-4 activator]), hence rescuing cells destined to die by apoptosis (Reddien et al., 2001; Hoeppner, 2001). This is reversed upon expression of the corresponding wild-type engulfment gene in the phagocytic cell (Reddien et al., 2001). But engulfment mutations in otherwise wild type, or *ced-3* null worms do not enhance cell survival. It suggests that the effect is dependent on the induction of at least low levels of CED-3, and plays an important role only when the cell-death machinery is compromised (Reddien et al., 2001).

Hence, it can be surmised that CED-3 is initially induced to a level sufficient to induce morphological changes and the exposure of eat-me signals, but not sufficient to ensure the completion of apoptosis. The engulfment machinery then positively feeds back to guarantee death of the cell (fig 1). This means that cells in which only low levels of caspases have been activated (and undergone morphological changes) can still be rescued to form fully differentiated, functional cells (Reddien et al., 2001; Hoeppner, 2001).

Blocks in apoptosis and engulfment are non-lethal in laboratory-grown *C. elegans*, but can be detrimental in higher organisms (Ren and Savill, 1998) as well, to ensure proper functioning and control of apoptosis. More work has to be done to elucidate the details of the feedback mechanism in *C. elegans*, and to identify homologous pathways in higher organisms.

Dynamic relationship between phagocytes and cells committed to die

Other experiments have shown that not only do phagocytes determine the fate of apoptotic cells, dying cells can also affect the survival of phagocytes (Tepass et al., 1994). In *Drosophila*, apoptotic clearance is performed by professional phagocytes, which are differentiated from haemocytes. Mutants that lack haemocytes can still initiate and complete apoptosis. But apoptosis-incompetent mutants (due to elimination of important pro-apoptotic genes hid, reaper and grim) fail to produce phagocytes from

haemocytes, and do not express the CD36-like scavenger receptor Croquemort. Conversely, the induction of ectopic apoptosis results in an increased number of phagocytes (Zhou et al., 1995; Franc et al., 1999). Therefore, the differentiation of haemocytes into phagocytes in *Drosophila* is dependent on the presence of apoptotic cells.

CONCLUSION

Macrophage clearance of apoptotic bodies is crucial in higher organisms. It is efficient and non-inflammatory, hence limiting and controlling cell death. Although the exact details of apoptotic eat-me signals and the engulfment machinery have not been elucidated, PS, the PS receptor, scavenger receptors, CD14 and integrins play central roles. Drosophila and C. elegans provide useful models for work in this field. Recent evidence suggests the presence of a positive feedback loop between the cell-death and engulfment machineries. It is increasingly evident that complex interactions exist between phagocytes and apoptotic cells. Not only might phagocytes initiate and regulate apoptosis, apoptotic cells may also influence the fate of phagocytes. More work is required before we can better understand the nature of this relationship, and engineer possible therapeutic applications.

ABOUT THE AUTHOR

The author wrote this paper as a final term project while studying abroad at St Catherine's College, Oxford University. She will graduate this May from Johns Hopkins University, and will go on to Stanford University for her PhD in Immunology

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Leptin As Fat Orchestrator

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SUMMARY

This work aims to unify the disparate and sometimes contradictory studies on leptin: the"hormone of plenty." In the eight years since leptin's discovery, focused studies have anatomized leptin in pathological metabolic conditions such as obesity, eating disorders, immune challenges and measured its response to simple fluctuations of temperature or exercise level. This synthetic analysis of leptin's role as the somatic energy signal and energy allocator in disparate states of stress attempts to elucidate this enigmatic and pluripotent hormone. At the same time, leptin may help us understand the endocrine etiology of some of medicine's greatest challenges.

LEPTIN AS FAT ORCHESTRATOR

Understanding of leptin expression and action is still in its infancy. Delineating leptin's structure, production, ligand binding and transportation gives clarity to any attempts to place the hormone in pathological landscapes. In constructing a thorough, selective review of contemporary leptin literature, a clearer evolutionary portrait materializes of a hormone integral to energy remittance, regulation and response. Perhaps most significantly, further avenues emerge for deeper understanding of leptin.

The challenge exists to outline a clear picture of leptin amidst the noise of endocrine, environmental, nutritional and psychological determinants of obesity and related conditions. This task requires collaboration and cross-theorizing between the fields of nutrition, metabolism, immunity and endocrinology.

The debate is not limited within the biological mechanisms of leptin alone, however. A case study of leptin is integrally involved in two especially ardent debates: the etiology of obesity as well as metabolic-immune intercommunication.

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Leptin background

The 1994 landmark paper by Rayner and Trayhurn (2001) revealed that obese ob/ob mice exhibited elevated expression of the leptin gene but no circulating leptin whatsoever. Therapeutic leptin treatment appeared to circumvent the ob gene deficiency and reestablish metabolic balance in the ob/ob mice (Morio et al. 1999). Images soon flourished in scientific and popular media of a little mouse's transformation from fat to thin after leptin injection. Leptin still continues to fascinate lay dieters and scientists alike as reports indicate the hormone may act as a negative feedback mechanism of food intake and body weight as well as a stimulant of energy expenditure (Matsuoka et al. 1997).

Studies show that leptin levels can be predicted statistically only with four independent parameters: Body Mass Index (BMI, where BMI= kg/m2, a standard measure of health risk due to obesity), percent body fat, gender and glycerol concentration in the blood. Alone, none of these parameters are exact indicators of leptin and often underestimate obesity levels (Matsuoka et al. 1997). Biological elements of leptin cause and consequence can only be understood amonst a varied individual endocrinological landscape (Morio et al. 1999).

Hormone interplay

Endocrinology studies must be careful not to isolate hormone actions and cascades. The biological reality of endocrinology is an extensive reciprocity and codependence—at once puzzling and staggering.

One of the important supporting actors in the leptin story is neuropeptide y (NPY), secreted by cells in the gut and the hypothalamus and integral to metabolism, obesity, anxiety, depression, memory, circadian rhythms and endocrine action (Inui 1999). Neurons of the hypothalamic arcuate nucleus (ARC) secrete the peptide (Inui 1999). NPY treatments inhibit satiety, induce feeding behavior and chronically induce rodent obesity. Commonly detected throughout the CNS, this neuropeptide acts on a cellular level to modulate lipoprotein lipase activity level, insulin secretion and energy expenditure (Mantovani et al. 2001). Fulfilling its homeostatic role, NPY blunts leptin and provides insulin negative feedback in conditions of energy deficit. This orexigneic action is counterbalanced by cytokine action decreasing appetite and increasing energy expenditure (Inui

1999). In the opposite milieu, positive energy balance is achieved by NPY signals increasing appetite, lipogenic enzymes and reduced brown adipose tissue outflow (Inui 1999).

In many ways, NPY appears to be leptin's main endocrine adversary. NPY decreases with leptin treatment, and vice versa, in both fed and fasted test animals (Rayner and Trayhurn 2001). Locationally, both NPY and leptin receptors reside together in arcuate neurons. Leptin-receptor binding may affect appetite mainly via inhibition of NPY synthesis and release (Mantovani et al. 2001). In another framework, NPY might then be an actuator of leptin's alarm message.

Lack of the NPY gene as a sole mutation produces mice with normal weight, starvation-response and leptin response. NPY can not, therefore, substantiate the sole force behind energy homeostasis. Neither is it essential for leptin action. The dual mutations of the ob and NPY gene in mice, however, do attenuate obesity phenotypes (Elmquist 1998, Rayner and Trayhurn 2001).

LEPTIN: SETTING THE BODY'S FAT THERMOSTAT

A useful (albeit loose) construct for leptin action is as a weight set-point mechanism, guarding a unique individual equilibrium. Leptin conceptually adjusts the "body's fat thermostat" based on environmental conditions. Such a regulatory operation would explain the remarkable constancy of individual weight despite great variation in food intake and exercise through time.

Tenacious weight stability is evident in the challenge obese individuals face in maintaining weight loss. Often, reduced energy expenditure counters weight loss efforts (Ioffe 1998). And while dieting may improve obesity and diabetes, "normal glucose tolerance may be difficult to achieve or maintain" (Muzzin et al. 1996, p. 14804). Adipose tissue size and distribution is far from arbitrary and is in fact strictly regulated (Halaas et al 1997). Obese persons do not respond to greater and greater leptin levels. The apparent blunting of the leptin response is often explained by a leptin-resistance theory of obesity.

Injections of leptin theoretically set a lower, constant body weight target by reducing food intake. Rates of energy use are unaffected. Higher leptin infusion rates achieve weight reduction faster, but do not achieve a greater quantitative loss (Halaas et al.1997). This nadir appears to be the leptin-guided set point.

To a great extent, metabolic efficiency predisposes individuals to certain weight classes (Reidy and Weber 2002). Three different mouse obesity strains: NZO, DIO, A^y show very disparate levels of leptin resistance. NZO mice respond to extremely low intracerebroventricular leptin levels while DIO and A^y do not. Subcutaneous leptin application, on the other hand, elicited a response in DIO mice (although some research is contradictory) and not in the other two obese strains (Halaas et al. 1997). A transport obstacle of some sort might explain these obesity triggers. The details beg for explication. Also, these types of similar variations in leptin resistance and practical "set points" should be sought in humans.

Leptin effects are precluded in A^y mice by the downstream impairment of the critical receptor in the leptin cascade, Melanocortin receptor 4 (MC-4). The agouti protein is thought to competitively inhibit binding of the α melanocyte-stimulating hormone (αMSH) to MC-4. Disruption of the leptin neural pathway at this receptor was recently imputed in 5% of severe childhood obesity (Russell 2001). Neurons brandish MC-4 receptors, Ob-Rb receptors, as well as the MSH precursor, proopiomelanocortin (POMC) (Halaas et al. 1997). All of these are subject to abnormalities accused in obesity.

Fasting and leptin

The relationship between fasting and leptin merits further study. With this goal, studies have sought leptin measurements of animals in fasting states. Starvation precipitates a dramatic fall in leptin gene expression (though readily reversible) in mice and humans. The greater the initial leptin level, the steeper its decline with weight loss (Lord et al. 1998). Thus, leptin effects seem to work via relative changes rather than quantized levels. Also, leptin decreases are apparent long before any reduction of adipose tissue, and therefore can be considered independently. Lack of insulin response is discredited as an impetus for the fasting leptin drop by examining obese mice: the insulin declines in obese mice with faulty leptin (fa/fa or ob/ob) fail to retrench leptin levels (Rayner and Trayhurn 2001).

Energy expenditure

Does leptin increase energy expenditure? Marked differences in leptin-treated energy expenditure engender incongruous conclusions. Thermogenesis and oxygen depletion seem to increase with leptin treatment (Fruhbeck et al. 1998). Reduced food intake normally causes a parallel reduction in energy consumption. Leptin, in most cases, blunts this energy frugality. Ob/ob mice, however, experience an antithetical energy expenditure peak with leptin application. Leptin action is fundamentally different than either food intake or energy availability. Caloric intake increases both lean body mass and adipose tissue while leptin affects only the latter (Halaas et al. 1997).

Females and leptin

Female rats possess higher leptin levels at any body mass (Rayner and Trayhurn 2001). While human females test at the higher end of the leptin-level spectrum, leptin levels are similar for both genders when standardized by percent body fat. Boys and girls show similar leptin levels and rates of increase with age (Matsuoka 1998). Gender inequity appears only at mid-puberty (Morio et al. 1999), when it continues to rise in girls and decline in boys. An enigmatic question remains in brain differences; why is the percentage of circulating leptin emanating from the brain so much higher in females (41%) than in lean males (13%) (Wiesner

et al. 1999)? Many interesting suppositions arise aobut differences in male and female eating behaviors. Upregulated leptin mRNA in proportionally larger adipocytes of female may explain part, but not all, of the gender difference (Fruhbeck et al. 1998). The mystery of the sexes subsists.

Reproduction and Leptin

Leptin accelerates first estrus in normal mice and reverses ob/ob mice sterility (Rayner and Trayhurn 2001). Leptin's induction of luetinizing hormone (LH, a hormone active in sexual maturation) release is most potent just before puberty in normal rats. Naturally, the theory of leptin-driven puberty onset in humans is substantiated by the rise of plasma leptin with accumulating adipose tissue through childhood. The role of leptin in obesity-driven infertility is particularly interesting since diet restrictions resulting in weight loss restore neither fertility or normal leptin levels. The power and sustainability of the leptin signal is thus reaffirmed in communicating information to the hypothalamic-pituitary-gonadal (HPG) axis. Exactly how and why leptin is necessary for fertility and estrus cycling is still the subject of study (Stoving et al. 1998).

Both obesity and emaciation obstruct ovarian function. Amenorrhoea often precedes weight loss in human anorexics and affects certain female athletes. Interestingly, amenorrhoeic athletes lack the nocturnal leptin peak found in menstruating athletes of equal weight (Stoving et al. 1998). Leptin's nocturnal peak must pose characteristic importance, as evidenced by its noticeable absence in amenorreaic athletes. By extension, then, leptin cycling critically regulates the gonadal axis.

Pregnancy Anomaly

Pregnancy poses a natural and definitively unique environment of leptin action. Like the obesity conditions discussed earlier, pregnancy imposes weight gains and corresponding leptin increases. Leptin's satiating qualities are vitiated, in fact, by the *elevated* appetite observed in high-leptin pregnancies. A leptin resistant model similar to obesity might be applicable. Post-pregnancy difficulties in weight loss suggest a stubborn quality of leptin resistance once adopted. How does a normal pregnancy regulate such leptin sensitivity? The strategic distribution of gestational fat may play a role. Placental secretion of leptin is also little understood, as is leptin action in fetal development and weight determination (Fruhbeck et al. 1998).

LEPTIN AND OBSESSIVE FOOD BEHAVIORS

Obsessive food patterns are likely to be wired as behavioral routines in the hypothalamus. "It is not the path that we choose in life that determines our happiness, it is the way we walk it" (Pickard, as quoted in Mac Evilly and Kelly 2001, p 27). Obsessive behaviors are mediated via dopaminergic midbrain system of reward and habit formation. Leptin, as well, may play a fascinating part.

Anorexia: antithetical metabolic malady

Anorexia is a nefarious and pandemic malignancy accompanying infection, inflammation, cancer and depression. Modern outbreaks of anorexia nervosa (1% of young women) and bulimia nervosa (4% of young women) are devastating and costly illnesses. The average cost of an individual anorexic's hospital stay is $12,390. Societal and environmental causes explain much of the female susceptibility (96% of victims are female), but genetic and endocrine predispositions are likely to be imputed in coming years (O'Brien and Patrick 2001).

Energy shortages normally induce greater food consumption and reduced energy expenditure. What inhibits these responses in anorexia/cachexia? [Anorexia is loss of appetite observable in disease; cachexia, a type of anorexia, involves adipose and muscle degradation and anorexia.] The scientific finger of blame lands squarely on inflammatory cytokines. Theories of cytokine-induced anorexia address the possible default in neural controls of the glucose response (Inui 1999). IL-1β, IL-8 and TNF-α induce anorexia either directly via the CNS or peripherally by activating second messengers, such as nitric oxide and prostanoids, through the vagus nerve or brain vasculature. The anorectic effect is also amplified by cytokine stimulation of CCK, a known appetite suppressor (Inui 1999). CCK might elicit anorexia via NPY feeding inhibition. Carbohydrate foods stimulate platelet-poor plasma (PPP) serotonin (5-HT) (the so-called "feel-good" hormone) levels in normal adults (Vered et al. 2001). It appears anorexics lack this default carbohydrate PPP serotonin response to carbohydrate intake.

Endocrine and neuroendocrine genes are foremost in the focus of present study of anorexia (Kaye et al. 2000). As expected, anorexics show lower basal leptin levels than normal-weight subjects, in proportion to their reduced percentage of body fat. Moreover, diurnal leptin fluctuation is considerably blunted in anorexic subjects compared to control subjects, despite equivalent food consumption and absorption (as measured by postprandial insulin and glucose levels) (Stoving et al. 1998).

In anorexia, as opposed to starvation, leptin works against the gradient of energy balance by maintaining fat stores despite incongruent energy consumption and expenditure. The question remains: why is the counterregulatory mechanism not triggered (Inui 1999)? Shockingly, anorexics might experience a similar leptin resistance to that of obese individuals. In theory, leptin would become ineffective in conditions of anorexia. Supporting evidence is gleaned from aging experiments that report exacerbated leptin resistance due to calorie restriction (Jacobson et al. 2002).

Ill communication in signaling mechanisms may be at fault (Russell 2001). Perhaps low anorexic insulin levels are partially responsible for a leptin decrease. Effects of irregular eating patterns and nutritional status should be integrated in any workable theory (Rock 1999). Some more outlandish theories describe anorexia as an adaptation in the face of certain traumas or infections, or as a staged de-

fense against foods that might hinder the immune response (Fernandez-Real 1999).

Hypothalamic amenorrhoea is a criterion for diagnosis of anorexia (Stoving et al. 1998). Falling plasma leptin concentrations are thought to revert many victims to a pseudo-prepubertal state. Again, though, the difficulty in disjoining leptin and weight loss consequences reappears. Tangentially, reduced leptin causes parallel decreases in LHRH and gonadotrophin release, which is distinctly detrimental to hormonal reproductive function (McCann 2001). Perhaps anorexics' propensity for irreversible bone density damage (Rock 1999) is additionally demonstrative of leptin reversion.

Weight gain in anorexics invokes disparate leptin responses even with similar weight, age and BMI (1998). Variation might reflect varying stages of nutritional distress or individual patterns of leptin and weight recovery. As a suggestion for clinical eating disorder treatment, Stoving et al. propose manipulation of leptin levels to restrain accelerated rates of weight recovery (1998). Detriments are likely to be subtle and insidious and more study should attempt to delineate the full picture of anorexic recovery. Can instructive parallels be drawn from weight gain in these subjects to weight gain in obese persons? Or perhaps weight *loss* in obese persons?

STRESS AND METABOLISM

How is stress linked to metabolism? A known consequence of the fight or flight stress response is the diversion of resources from activities necessary for long-term survival (digestion, immunity, reproduction) to those necessary for immediate survival (muscles, eyesight, alertness). The main stress hormone group, glucocorticoids (GC), is mechanistically linked to fat tissue in that it elicits differentiation and proliferation, possibly on a site-by-site basis. Moderate GC treatment normalizes leptin responses, while excessive treatment induces overfeeding and obesity despite high leptin levels. Excess and resistance of leptin are mirrored and likely related to glucocorticoid excess (Bjorntorp 2001). The inverse relationship between plasma levels of cortisol and leptin in situ raise theories of mutual inhibition. Some studies present stress's potential to diminish leptin patterns (Bornstein et al. 1998, Kain et al. 1999).

Changes in consumption resultant from stress, either positive or negative, vary according to individual and circumstance. "Stress-eating" comprises several overeating responses to emotional stress. Distress is ameliorated, in theory, by centrally- released opioids after food consumption. Explicating the phenomenon, most bearers of restrained food attitudes are female and the most salient focus is fat intake. The nebulous construct of stress-eating might be a manifestation of an internal contest between leptin and neuropeptide y, with the latter's triumph driving appetite (Bjorntorp 2001).

Leptin as signal of plenty and stress responder

The combination of stressors and free radical accumulation incurs harm on the body. A new understanding of leptin as deleterious over long time periods, sows the concept of accrued damage from a contiguous or exaggerated stress response. Even psychological manifestations of depression are most likely selected as stress responses compelling an individual to "lay low" and avoid risk (Wright 1994).

The most precise characterization of leptin, however, is as orchestrator of the body's energy furnaces, seamlessly guiding crescendos and lulls in response to stresses such as excessive cold, to burns, to exercise in mellifluous unison (Reidy and Weber 2002). Independent of fat changes, leptin triggers events to maintain homeostatic energy balance in the short-term. In times of energy crisis and imbalance, leptin's signal is most clearly heeded. Studies of Siberian hamsters report attenuated leptin response in short-photoperiod (wintering) animals exposed to energy deficit as compared to long-photoperiod animals. Regardless of fat content, leptin declines most precipitously in the first 24 hours of fasting. The dramatic and rapid variation in leptin levels is demonstrative of its responder role (Oritz et al. 2001). A corollary of this theory would parallel the body's dramatic response to night eating syndrome and anorexia to fasting conditions.

Leptin selection – evolutionary perspective

The potential for leptin to reduce adipose tissue in obese individuals has received romantisized attention (and will presumably continue to) as a product of an anti-obesity gene or- better still- a magic diet pill (Unger et al. 1999). It remains extremely unlikely that leptin's evolutionarily purpose is the readjustment of adiposity in overfed states. On the contrary, the "thrifty gene" theory purports a selective advantage for organsims retaining fuel reserves to safeguard against famine in conditions of alternating food availability. The potential fitness gains from food intake are recognized and encouraged by both serotenergic and appetitive mechanisms.

Responsible science demands that future research look not only at single individuals in time, but throughout a phylogeny to examine adaptive potential, causes and consequences. Famine and starvation have been eternal and inexorable constraints; obesity, as a modern epidemic, has had little time to be selected against. Temporary energy stockpilings have been selected across diverse organisms, from the penguin before molting to the desert rat in preparation for summer famine (Unger et al. 1999). High-energy phosphate bonds in fat molecules hold great metabolic potential (Nonogaki 2000).

Leptin resistance may have adapted in present-day conditions of over-abundance to prevent the dramatic leptin actions in animals possessing sufficient fat stores. Obesity conditions, in one sense, are "`mal-adaptations' of actual lifestyle to our genome" (Fernandez-Real & Ricart 1999). The biochemical reasons for low-concentration efficacy could include a limited CNS entry, increased competition

for binding, etc. The theory also enucleates lack of leptin correlations in end-stage renal disease cases (Fruhbeck et al. 1999). The kidney failure obstructs the key regulation of leptin levels by clearance.

Leptin, nutrition and immunity

As part of the stress response, leptin incites inflammation in the immune response. Leptin mimics the exaggerated responses often induced by infection and autoimmunity and may foster heightened inflammatory responses. This puissant hormone increases IFN-y and IL-2 production, while completely inhibiting regulatory IL-4 (Lord et al. 1998). Cytokines as well as naïve and memory T cells also operate within leptin's purview. The culminating effect is inflammation, which is then blocked from feedback inhibition (Drazen et al. 2001).

Impaired nutrient utilization is often couched as a corollary of immune challenge. More likely, there is cross communication. Decreased energy ingestion and uptake is at first counterintuitive in a state of immune challenge as it challenges the pathogen for energy sources as well as the host. Leptin's involvement, if further understood, would reveal much of this clandestine activity.

Abnormalities in the aeges of metabolism and immunity are mutually dependent and exacerbating, with the TNF-leptin pathway enabling this cross-communication. Effective campaigns against nutritional deficit, disease and infection cannot be waged in isolation. A central question lingers concerning whether cytokines or leptin act as the main regulator, or is mutual feedback the only means of matching energetic needs. Aging, cancer and other diseases are not caused by leptin, but perhaps by resistance to or diminution of the hormone (Mantovani et al. 2001).

The immune response is quite energetically expensive, increasing oxygen consumption and body temperature. Energy reserves are required to mount antibody response to specific pathogens. Two countering theories explain starvation-induced immunosuppression via energy unavailability or as a stress response. Evidence suggests, surprisingly, that leptin levels respond to the energy reduction of fasting, and not the stress response of the HPA system. Siberian hamsters with short photoperiods (usually incurring low fat and leptin levels) respond to leptin treatment with increased immune function (as measured by IgG concentrations) and increased food intake without any change in cortisol level. Leptin's indirect effect, then, is increasing energy fuel through increased consumption and improving immune preparedness (Drazen et al. 2001).

Obesity Intervention

The idea that so enthralled scientists and dieters alike is that leptin might mitigate appetite and promote weight reduction. In very rare instances of mutations preventing leptin production, this can be true. Large leptin doses decrease food intake in mice and rats, especially when applied intracerebroventricularly to act at the hypothalamic command center. For the most part, however, exogenous leptin does not reduce intake and fails as an easy cure-all for obesity.

Still there may be means of rational and promising obesity intervention through diet, nutrient and exercises changes. Optimizing fuel energy release as measured by thermic effect of food (TEF) may be possible by consumption of regularly timed and high-protein meals. Sibutramine and B_3-adrenergic agonists are some propositions as boosters of basal metabolic rate through sympathetic action (Pinkney et al. 2002). UCP action and TAG/FA cycle regulation may affect individual energy expenditure, and may be useful in understanding and treating obesity (Bjorntorp 2001). Vagally mediated hyperinsulinaemia may be quieted via sympathomimetic and anti-cholinergic chemicals. Octreotide successfully reduced hyperphagia in cases of childhood hypothalamic obesity (Pinkney et al. 2002).

Hypothalamic impairment would prevent most NPY, leptin, serotonin and noradrenaline actions. Possible pharmacological targets include: NPY (Inui 1999), melanocortin-4 receptor agonists, galanin antagonists, cytokine agonists and serotonin agonists. Serotonin enhancers, fenfluramine and fluoxetine, show some success in a specific type of genetic obesity (Pinkney et al. 2002). Lipase inhibitors require specific diet restraints, but may prove effective. Cortisol might harvest another target for centrally-obese persons suffering from raised cortisol levels. The approach of corrected hypercortisolaemia achieved normalization of associated maladies in Cushing's syndrome subjects (Bjorntorp 2001). Obese patients with naturally- low leptin levels appear to respond most acutely to exogenous leptin treatment. For maladies related to low body weight, anti-leptin antibodies and molecules aimed at impairing leptin have shown success in clinical weight gain (Bryson 2000).

So much of the obesity problem remains shrouded in complexity and mystery. Where is the malfunction? Scientific focus must remain on regulatory systems, and not just symptoms and comorbidities of obesity. Although a specific leptin receptor mutation causing obesity has been disproved, gradations of receptor affinity remain a possibility. Handicapped leptin function is either a causal, resultant or conditional characteristic of obesity.

Obesity may even represent a consequence of the neuroendocrine stress responses. Is cortisol responsible for leptin resistance and ensuing obesity (Bjorntorp 2001)? If not, what is the cause of the enigmatic leptin resistance? Since leptin infusion itself is unlikely to produce the dramatic weight loss initially hoped, a more pragmatic approach would be the pursuit of causes and mechanisms of leptin resistance rather than addressing purely leptin underproduction. Additional factors, like the recently discovered resistin (SCM 200), are likely to obfuscate metabolic mechanisms further. Leptin offers promise in regulating and ameliorating metabolic disorders from diabetes to wasting disorders from HIV and cancer. Hormonal understanding and treatments are only one tool against the obesity epidemic, and should be combined with an arsenal addressing mod-

ern lifestyle issues of consumption, exercise and stress. Leptin is not a panacea.

Future implications: Leptin has gained weight

By all accounts, the leptin story is incomplete. Extensions of the quests undertaken by the Human Genome Project will elucidate the precise polymorphisms of hormone production, receptors and action related to obesity. Work is currently underway to sequence the critical 5' promoter of the glucocorticoid receptor gene, for example (Bjorntorp 2001). Quantitative Trait Loci (QTLs) will enable the pinpointing of correlations and functional significances affecting complex traits. Moving past simple mapping and identification of candidate genes is critical to explore pleitropic actions of genes, epistasis and environmental interplay (Phillips et al. 2002). Proteomics promises the revelation of additional leptin regulators running the gamut from marginal to essential (Russell 2001).

Leptin, as the signal of plenty, pronounces the good news of fuel abundance to many aeges, including the reproductive system (and thus enabling normal reproductive development and female cycling) and the immune system (and boosting the immune response). In affecting food intake and storage, leptin assures energetic intake balances with expenditure. The pluripotent hormone is more than a steadying adipostat, however, but also a dramatic responder to stresses of all kinds. Leptin functions simultaneously as tonic regulator and emergency responder. In times of metabolic and immune stress, leptin reallocates resources to the most critical activities.

Leptin takes center stage in the current struggles to elucidate and starve the American obesity epidemic. Amid wild speculation and outlandish assertions, is the core truth that leptin plays a critical role in weight regulation. Complexities of metabolism and genetics have just gained weight.

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Molecular Dynamics as a Bridge: Fundamentals, Methods, and Current Research

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ABSTRACT

Molecular dynamics (MD) is a widely used tool in condensed matter physics, as well as other disciplines ranging from chemistry to high-energy physics. In MD, one integrates the equations of motion – Newton's second law for classical particles – directly, invoking no approximations. To do so requires an interaction potential energy or forces between atoms. I will discuss both integration schemes and potential types. MD potentially bridges two length scales – macroscopic and atomistic – and also links experimental results with theories. This will be emphasized through a discussion of modern research in solid-state physics, with each research application highlighting a different type of interaction potential. I will discuss fluid flow briefly and highlight some other applications of Lennard-Jones potentials, surface growth using a Stillinger-Weber potential, and defects in silicon using tight-binding potentials. I give some references regarding density-functional theory calculations of these defects.

A different avenue of modern MD research is in the method itself rather than its application. Much research is towards developing so-called *acceleration methods*. By taking advantage of the physics of condensed matter systems, acceleration methods have been proposed which extend the time scales accessible by MD by orders-of-magnitude in many cases. In this article, I will focus on A. F. Voter's methods, giving their motivation, algorithm, and some derivations.

In short, this article explain why MD is useful and where it has been used, it gives the fundamentals necessary for understanding a MD simulation, and discusses research into MD methods themselves, namely Voter et al.'s acceleration methods.

Potentials

To *accurately* propagate the system, we must have an *accurate* interaction potential. This potential gives the potential energy as a function of the atomic positions and velocities. Details on existing potentials are given, as well as some general ways of deducing potentials.

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Common potentials:

One can generally group potentials into three basic categories: classical potentials, tight-binding potentials, and density-functional calculations, in order of increasing computational complexity. Representative examples are discussed: Lennard-Jones (LJ), MEAM, and Stillinger-Weber (SW); empirical tight-binding (TB); and density-functional theory (DFT). LJ considers interactions between pairs of atoms; MEAM adds corrections, beyond pairwise interactions, based on the local geometry; TB and DFT add quantum mechanics at increasingly sophisticated levels. Each potential will be discussed later in terms of a particular research application.

Regardless of the potential, deriving its form requires assumptions, or the form of the potential may just be guessed. In optimizing a potential's accuracy, parameters are adjusted (like the balance between attractive and repulsive LJ parameters – see below) so the potential reproduces quantities such as lattice constants and defect energies obtained from first-principles calculations or experiment (Baskes, 2001).

Efficient potential calculations – see (Rapaport, 1997) for more information:

Once the potential is obtained, it must be computed repeatedly in a MD simulation, a $O(N^2)$ or worse calculation, where N is the number of particles, rendering simulation of large systems impossible. A O(N) calculation is achieved in practice by one of two methods:

1. The "cell method" – This method divides the system being simulated into cells, each with a linear size larger than the interaction cutoff r_c (Figure 1), where r_c is a value such that atoms farther apart than this value essentially do not interact. Now one needs only to compute interactions between atoms in the same and adjacent cells (Figure 1), causing a speedup to O(N) for even moderate size systems.

2. The "neighbor list method" — In fact, only 16% of the atoms, on average, included in the cell method are within a distance of r_c (Rapaport, 1997). Hence we keep a list of all "neighbors" of an atom. This is actually an $O(N^2)$ calculation. Fortunately, one can code this efficiently and only compute it only once every many time-steps since there is an upper bound to how fast things are moving, effectively making the overall system an efficient O(N) calculation for most system sizes.

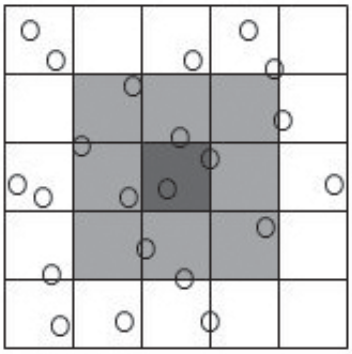

Figure 1. Two-dimensional illustration of the cell method. The dark gray cell needs only to check the light gray cells – its nearest neighbors – because all other atoms must lie outside of the interaction cutoff range r_c, the range beyond which no particles interact.

Position integration algorithms.

Some details of potentials and their calculation are now familiar. With the potential and initial conditions Newton's second law can be integrated for all the particles. The verlet and predictor-corrector (PC) methods are common for performing the integration.

The integration algorithm usually does not need to be extremely accurate. Because an extremely slight position displacement at any time (or an equivalent round-off error) can cause huge differences in the atom's trajectory at all later times after a certain time, only quantities which are insensitive to exact trajectories "matter." This is not particular to MD, but is characteristic of the natural process itself.

The verlet method gives positions at a short time Δt after the time corresponding to the supplied positions. Each is derived in a straightforward manner from the Taylor expansion, in time, of the atomic coordinates. The formula for the verlet propagator is (Rapaport, 1997):

$$x(t+\Delta t) = 2x(t) - x(t-\Delta t) + h^2 \frac{d^2x}{dt^2} + O(\Delta t^4)$$

The verlet propagator is commonly used for its simplicity and tendency to conserve energy.

The PC methods are more accurate than verlet, but they are not used as frequently as the simpler verlet-class propagators. The primary advantage of the PC method over the verlet-like algorithms is in the ability to change Δt on the fly, which may be useful in systems where one set of particles inherently move faster than others. PC is also useful when constraints (on, say, bond length or angles) are placed on the system (Rapaport, 1997).

The PC method predicts positions based upon Adams-Bashforth extrapolation, which is exact if they follow monic polynomials. After prediction, correction is made via a different set of formulae. For the (relatively complex) equations see (Rapaport, 1997).

Better (Faster!) Molecular Dynamics.

Although MD is an increasingly mature field, there are still continuous advances in methods. Voter et al. have developed several methods for increasing MD's performance by many orders of magnitude. Each method requires some assumptions – usually forms of transition state theory (TST) (Voter, 2002 or Lombardo, 1991) – on the nature of transitions. However, the assumptions are minimal and their validity can be checked.

There are three common families of acceleration techniques, namely parallel-replica (PR or par-rep), temperature-accelerated dynamics (TAD), and hyperdynamics. The families can be utilized simultaneously for multiplicative performance boosts. Voter gives an excellent, accessible review concentrating on these acceleration methods (Voter, 2002).

The limitation that keeps one from simulating long time scales is the fact that MD is a multi-scale problem (for most solid-state systems). Specifically, one must use a small enough integration time step to reproduce the dynamics of the fast vibrational modes. Since these vibrations occur on the order of 10^{13} - 10^{14} times a second, the time step for accurate integration must be on the order of femtoseconds. A typical time step falls in the range of 1-5 fs.

On the other hand, transitions occur infrequently; time scales between interesting transitions range from picoseconds (quickly diffusing surface atoms) to seconds (dislocation motion under shearing (Haasen, 1996)). One can conceive of watching interesting behavior for minutes or hours (for example, in crystal growth), however the longest MD simulations can now run for only microseconds. It must be kept in mind that the acceleration methods discussed below only apply to *infrequent event* systems, systems in which the time for transitions between 'sites' or 'structures' is much longer than the vibrational period.

Parallel-Replica.

When running MD simulations on parallel computers such as clusters one can separate the calculation so that each processor deals only with a portion of the atoms. Thus increasing the number of processors one can increase the size of the simulated system. However, each processor must have many atoms associated to it or else the communication time between processors, rather than the computational time, will be the performance bottleneck. While a spatial decomposition of the problem is conceptually easy, there is no straightforward way to decompose the simulation in time because each set of positions requires the previous positions.

To solve this problem, Voter created the parallel-replica (PR) algorithm (Voter, 1998). The only assumption of PR is that the probability of a system escaping a site (transitioning from one potential minimum to another) between times t and t+dt is given by:

$$p(t)dt = k\exp(-kt)dt \qquad \text{(Eq. 1)}$$

where k is the rate constant.

This is satisfied by infrequent systems when the sites are uncorrelated (Voter, 2002). Here uncorrelated means

that once a system enters a new potential well (crosses a saddle point in the potential energy surface) it has no "memory" of the site it just arrived from; that is, the dynamics after the crossing are not affected by how it got to that site.

The PR method has been extended to work when the probability distribution function has a different form than Eq. 3 (Shirts, 2001). However, one must be very careful in this case as it indicates that a "state" is composed of several potential minima or that the runs on each processor are not independent (see below).

With this assumption, the parallel-replica procedure can be shown to give the same dynamics as a system simulated with non-accelerated dynamics in the sense that the sequences of transitions from state to state are reproduced in the long time limit. I will present how the procedure works; for a proof, see (Voter, 2002).

To start a parallel-replica simulation, identical systems are set up on multiple processors. The number of processors can often be very large and consist of many different types/speeds of processors. Now, to ensure that each processor is simulating independent trajectories, the dynamics run at finite temperature with momenta randomized every few time steps.

All the processors now run dynamics until the system on one of the processors escapes from its current state. The time elapsed on each processor is summed and this is identified as the total time spent in the state from which the system has just escaped. The processor that computed the escape continues to run for a certain length of time such that after this additional time there is no memory of the state from which the system escaped.

The PR method solves, to some degree, the process of parallelizing MD in the time domain. It has been often utilized (Zagrovic B, 2001; Shirts, 2000; or Birner, 2001). Still, much the performance of the individual processors can be increased. Hyperdynamics and temperature-accelerated dynamics are two solutions to this problem and are discussed next.

Hyperdynamics.

One (justified) way to view the time evolution of a system is as a succession of vibrational and transitional 'events'. This is illustrated in Figure 2; the system oscillates in this potential well for a certain time, and then an increase in local energy pushes the system over the transition barrier to a new potential well (Figure 2a). Remember that this is a high-dimensional potential well; forgetting this can misguide intuition.

Using this picture of dynamics, one can imagine speeding up the simulation by "filling in" the wells (Figure 2b). However, to do this one must be able to decide (automatically) whether the system is close to the bottom of the well or near the transition point, since the potential should be filled in more or less, respectively. Even assuming that one can fill in the wells with some technique, one must map the dynamics of the boosted system back to the non-accelerated systems. Voter et al. describes this process in (Voter, 2002).

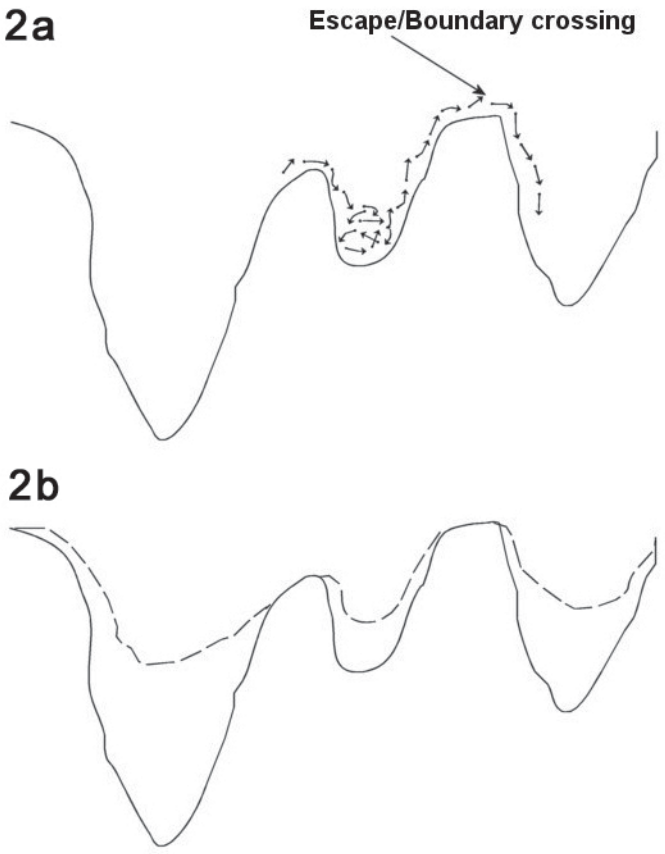

Figure 2. Viewing dynamics as a sequence of vibrational and translational events – applicable to many condensed matter systems – and the relation to hyperdynamics. a. The particle oscillates in its potential minimum then the energy of the system is temporarily increased, pushing the system over an energy barrier. b. By decreasing the depth of the potential wells, the dynamics are sped up since less energy is required to cross the barrier.

As a last note, the average boost factor for hyperdynamics is given by: $\frac{t_{hyper}}{t_{MD}} = \left\langle \exp\left(\frac{\Delta V}{k_B T}\right)\right\rangle$ with k_B Boltzmann's constant, T the temperature, and ΔV the increase in potential energy from the bias potential. This implies that we receive an exponentially bigger boost as the temperature of interest lowers. Hyperdynamics has not found nearly as much success as other acceleration methods due to technical difficulties in filling in the potential wells.

Temperature-accelerated dynamics.

The temperature-accelerated dynamics (TAD) method has been more successful than hyperdynamics to date. This method is related to hyperdynamics in that one increases transition frequencies by making the energy barriers easier to go over. Rather than changing the relative energetics, however, TAD simply raises the temperature of the system.

As with hyperdynamics, the accelerated dynamics will not directly correspond to the non-accelerated dynamics; rather, by some *a priori* assumptions, the dynamics are mapped to the lower (correct) temperature dynamics. In place of a usual MD simulation, *basin-constrained* MD (BCMD) is performed in which the system is evolved until a transition occurs. After this is detected, the trajectory is reversed, sending the system back into the basin from which it had just escaped. The saddle point is calculated using, say, the nudged-elastic band method (Henkelman, 2000) and stored. This continues until a specified certainty is reached that one possesses enough information to properly extrapolate the dynamics to lower temperature.

Harmonic transition-state theory (HTST) – the assumption placed on the system in this method – states that the probability distribution for first escape times for the basin in consideration is given by Eq. 1, with

$$k = \nu_0 \exp\left(-\frac{E_a}{k_B T}\right) \qquad \text{(Eq. 2)}$$

where E_a is the energy barrier the system must go over to escape from the basin, and ν_0 is some frequency characterizing how often the system vibrates or attempts to leave the basin (the *attempt prefactor*-).

Note that these equations explain why raising the temperature changes the dynamics; changing the temperature does not affect merely the magnitudes of each rate constant, but affect also the relative magnitudes (hence speed up one transition more than another). To extrapolate the transition time from high to low temperature

$$t_{low} = t_{high} \exp\left(E_a\left(\beta_{low} - \beta_{high}\right)\right) \qquad \text{(Eq. 3)}$$

is used (Figure 3), where t_{low} is the time at the low temperature and t_{high} is the time at the high temperature; $\beta = \frac{1}{k_B T}$, and E_a is the activation energy computed by nudged-elastic band or a related method (Henkelman, 2000).

To derive Eq. 3, change variables in the probability distribution (Eq. 1) to $x = kt$. Then

$$p(x)dx = p(t)\frac{dt}{dx}dx = k\exp(-kt)dx\frac{dt}{dx} = k\exp(-x)\left(\frac{1}{k}\right)dx = dx\exp(-x)$$

Hence $k_{low}t_{low}$ has the same probability distribution as $k_{high}t_{high}$, and

$$t_{low} = \frac{k_{high}t_{high}}{k_{low}} = t_{high}\exp\left(E_a\left(\beta_{low} - \beta_{high}\right)\right) .$$

This relation is illustrated graphically in Figure 3. Here it is clear that a transition that is not the shortest at high temperatures can be the shortest at a lower temperature – this is why *basin-constrained* dynamics must be performed. The transition with the shortest time for escape (at low temperature) is selected as the transition corresponding to the low-temperature dynamics and the system is updated. The BCMD is started again from this new site. To be confident, with certainty δ, that the current shortest time t_0 (at low-temperature T_{low}) is correct, the system must run in BCMD for a time

$$t_{stop} = \frac{\ln\left(\frac{1}{\delta}\right)}{\nu_{\min}}\left(\frac{\nu_{\min}t_{low,short}}{\ln\left(\frac{1}{\delta}\right)}\right)^{\frac{T_{low}}{T_{high}}}$$

where ν_{min} is the minimum attempt prefactor (see Eq. 2) which can be chosen manually, using reasonable guesses; t_low,short is the shortest found time (extrapolated to low-temperature) (Voter, 2002).

Using this technique, one boosts the simulation speed by a factor of hundreds when interested in low enough temperature systems (a couple hundred Kelvin). Recently, Voter proposed a TAD enhancement (Montalenti, 2001) in which one considers the total time spent in a state (previous and current visit). This requires a good way of telling if two states are the same, within symmetry, a topic just being explored in condensed matter systems (Jiang, 2003 and Machiraju, 2003). With this improved TAD method, the average boost factor for each state becomes $\exp\left(E_{\min}\left(\beta_{low} - \beta_{high}\right)\right)$ with E_{min} the minimum barrier for escape from the state in consideration (Montalenti, 2001).

I briefly mention *on-the-fly kinetic Monte Carlo* (OTFKMC) simulations. KMC uses a user-specified list of transition rates and, by sampling from the rate list, propagates the system in time. KMC requires *a priori* knowledge of the transitions and their rates, which is problematic. OTFKMC computes the list of transitions by MD simulation (Voter, 2002) or other means (Henkelman, 2003) as the simulation progresses. When the system returns to a state enough times, one assumes that the rate list is nearly complete and uses KMC. To see applications of TAD and OTFKMC, see (Sprague, 2002 or Montalenti, 2002 or Montalenti, 2001).

Recent simulations

Lennard-Jones potential – fluid flow and biophysics

Now I focus on current simulations performed using MD, starting with Lennard-Jones (LJ) potentials. LJ potentials are *pairwise* interaction potentials. That is, the total potential energy of a system is just a sum over all possible pairs i,j of atoms of a potential energy function

$U\left(\left|\mathbf{r}_i - \mathbf{r}_j\right|\right)$, where U is a function only of relative atomic positions of two atoms. These interactions capture the essence of many systems, and produce quantitatively the behavior of noble gas (say argon) liquids and gases in many cases – the noble gases were historically very important for the LJ potential (Collings, 1971, for example).

In the LJ potential there are two parameters: one parameter governs the strength of the attractive interaction and one of the repulsive interaction; equivalently one can use a parameter as the potential minima depth, and another as the atomic separation for the potential minimum (σ and ε respectively below). The basic formula for calculating the (LJ a-b) potential is $U\left(d_{ij}\right) = 4\varepsilon\left(\left(\frac{\sigma}{d_{ij}}\right)^a - \left(\frac{\sigma}{d_{ij}}\right)^b\right)$.

Usually a=12 and b=6; if this is the case the potential is generally just denoted LJ (rather than LJ 12-6). In Figure 4 one can see the strong repulsive core at short ranges and the weak attractive tail. The a=12 parameter is fairly arbitrary (fortunately some physics is relatively insensitive to the choice of a) and the value of b=6 comes from Van der Walls attraction of non-polar substances.

In addition to the noble gases, the LJ potential is still used as part of the total potential in biological applications (Schleyer, 1998 or Brooks, 1983). In these situations, long-range interactions are Coulombic and LJ, and short-range interactions (bonded atoms) obey Hooke's law. In testing new methods, low dimensional LJ model systems are often used. These systems have the advantage of giving qualitatively complicated and atomic-like potential surfaces as well as, in some cases, being analytically solvable. This, coupled with the quickness of their simulation, gives rise to LJ's usefulness in testing models.

Moreover, the potentials occasionally find use in condensed matter outside of biophysics. Despite the extreme simplicity of this model, it shows many complex phenomena characteristic of condensed matter systems (phase transitions, complex energy landscapes, infrequent events) and hence is studied to see the simplest examples of these phenomena (Scopigno, 2002 and Fabricius, 2002). A systematic study of large numbers of clusters of particles interacting via LJ yields interesting results on the dynamics and structures of these clusters, while suggesting general features that hint at features in real clusters (Doye, 1999). Also, when extremely large numbers of particles need to be simulated yet a continuum approach is not applicable, such as in some fluid flow problems, LJ is attractive. Millions of particles can be simulated for many time steps.

As an example, Berthier and Barrat (Berthier, 2002) have studied mixtures of sheared fluids using a LJ potential. Two types of particles are implemented via particle-type dependent σ's and ε's in the LJ potential. They are able to investigate velocity distributions, viscosity dependence on shear rate, and structure factors for the system at different temperature, spanning liquid, supercooled, and glassy states. Hence, they investigate this *macroscopic* system at an *atomistic* level of detail, bridging these length scales as promised in the introduction. They also correlate theoretical predictions (using a "mode-coupling approach") with the simulations, demonstrating another of the bridges discussed at the start. Others have used LJ to study fluid flow as well – for a flavor, try (Bruin, 1998 or Laredji, 1996).

Classical potentials (beyond Lennard-Jones)

The LJ potential does not include how the environment (nearby atoms) affects the pairwise interaction. That is, the potential depends on terms involving three or more relative positions, but LJ ignores this. Extra terms are necessary to accurately simulate many systems.

Typical potentials that incorporate these many-body interactions (in very different ways) are modifications to LJ to include explicit angular dependence, Stillinger-Weber (SW) (Stillinger, 1985), and the modified embedded atom method (MEAM) (Daw, 1984). Recently, MEAM has been used to tackle a large variety of systems; largely these have been surface diffusion/surface growth phenomena.

Lee et al. (Lee, 1998) and (Baskes 1997) (and many others) have studied *ad-dimer* (two atoms deposited on top of a surface) diffusion on silicon surfaces. *Adatom* (one deposited atom) diffusion was studied earlier (Roland, 1992). Here I will describe results garnered by Gawlinski and Gunton (G&G) for molecular-beam epitaxial (MBE) growth of Si(001) surfaces (Gawlinski, 1987), a simulation which involves diffusion of adatoms and ad-dimers as well as *coalescence*: the clusters meet and 'stick,' forming islands. G&G employed a SW potential for the simulation.

Some investigations have looked at the detailed mechanisms of the diffusion of these small clusters and coalescence of these. G&G chose to discuss only the *morphology* (basic shape) of the surfaces grown and to not discuss the exact mechanisms, since classical potentials may not properly incorporate important effects.

In MBE, atoms are deposited (in this case as single atoms) from the gas phase onto the crystal surface. G&G were inspired to simulate this process, in part, by the experimental discovery of an epitaxial growth transition temperature. Above this temperature the atoms, after striking the surface, have time to diffuse around and find globally stable (bulk-like) states; below this temperature, however, new atoms are deposited close to already deposited atoms before the deposited atoms can relax. This leads to amorphous (no local order) surface grown on the bulk phase (Gawlinski, 1987). One of the simulation goals was predicting this transition temperature; they succeeded in finding a temperature in good agreement with theory (Gawlinski, 1987).

In an ordered, layered crystal structure, the density of atoms as a function of some coordinate should have oscillatory behavior with sharp peaks. In amorphous structures, the density should be spread out relatively smoothly. Fig-

ures from G&G (Gawlinsky, 1987) are reproduced as Figures 5a,b. Figure 5a is the density of deposition occurring above the epitaxial transition temperature; Figure 5b is the corresponding density below the epitaxial transition temperature. Clearly, the top layers represented in Figure 5a are well layered, and in Figure 5b they seem more random. This case forms an excellent example of MD forming the third bridge I mentioned in the introduction, that with experiment.

While it is possible to compare simulation results with experiment, one must be extremely cautious in several regards. First, a simulation is only as accurate as the potentials used, leading to the cautious interpretation above (no detailed mechanisms). Moreover, in order to run this simulation (in 1987, even!) the deposition rate of incoming atoms had to be increased to extremely unrealistic values. If the time between atomic depositions should match experiment, it is likely that no depositions would have occurred in the time scale simulated. With this is mind, however, *something* must be going correctly, or the simulation would not have reproduced experiment.

Tight-binding potentials and density functional theory

While purely classical potentials have worked extremely well in some situations, often quantum effects are important. Both tight-binding (TB) and density-functional theory (DFT) methods incorporate quantum mechanical effects at some level. TB incorporates these at a much less accurate level than DFT, but TB is a couple orders-of-magnitude faster to calculate. Moreover, TB can scale linearly with system size in some cases by exploiting the fact that the calculation is essentially diagonalizing a sparse matrix (Galli, 1998 or Klimeck, 2002). DFT is also widespread in the literature, including the problem of silicon defect clusters discussed below (for example, Kohyama, 1999; Estreicher, 1998; or Windl, 1999). I focus on TB.

TB has found use in cluster and defect structure and defect diffusion in some materials (Arai, 1997 or Jansen, 1988). In particular, it has been used to simulate *interstitial* (extra atoms inserted between lattice sites) and *vacancy* (atom removed from lattice site) clusters. Also, groups have studied more extended structures such as the {311} defect in silicon (Kim, 2000). This is by no means an exhaustive list.

Many studies of defect clusters in silicon are not MD simulations, but only energetic calculations, especially in DFT. In investigations such as these, initial structures are guessed and then the system is locally minimized (Estreicher, 1998). The downside to this is the guessing. Quite unintuitive structures are typical (Richie, 2002) and missed by simple guessing. A few people (Wilkins, private communication) are advocates of performing “cheap” TB or classical MD to explore a problem and improve guesses for structures and transition pathways, followed by DFT energy calculations to confirm results.

Colombo reviews TB results of silicon defects in (Colombo, 2002). Naively, it may seem that when an interstitial meets a vacancy, they should annihilate, leaving only bulk silicon. This state is energetically favored, and, if given an infinite amount of time, the system will spend most of its time in the bulk state. However, experiments and experience tend to deal with finite time scales on the order of seconds. Colombo et al., (Tang, 1997) find that if an interstitial and a vacancy are placed within next nearest neighbors along the <110> direction (in which the dimer points) then annihilation does occur. If the vacancy and interstitial are instead separated by greater than next nearest neighbors they do not annihilate; they attract each other and form a stable interstitial-vacancy pair shown in Figure 6. The energy barrier for annihilation once achieving this state is greater than 1 eV, corresponding to a lifetime of hours for the defect at room temperature. This strange structure is a concrete example of why guessing structures and interaction mechanisms can fail (Richie, 2002 or Kim, 2000).

As a final note on this research, note that I have artificially assigned each problem domain to a type of potential. Often, these problems do not use the potential types I suggest. For example, hydrogen atoms effect on Si(001) surfaces have been studied with DFT (Dongxue, 2002); classical potentials have been used to study Si clusters (Birner, 2001); and TB potentials have been used for Ge diffusion on Si (110) surfaces (Katircioglu, 1994). The applications I have presented are a small fraction of all simulations performed – there is little limit to MD’s applications thanks to the lack of assumptions on the dynamics.

Conclusion.

This review does not cover all classes of research using MD – references are representative rather than exhaustive. However, it should have given the reader an idea of some typical applications – fluid dynamics, surface growth, and defect dynamics. Through examples, connections between

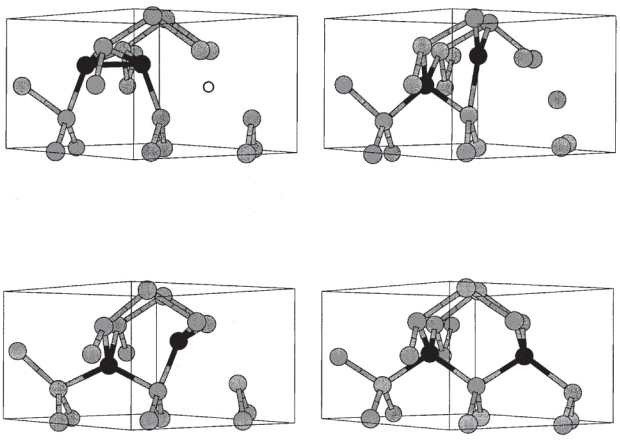

Figure 6. A stable interstitial-vacancy cluster (one extra atom, one missing atom) and its annhilation path. This unintuitive structure (top left) is higher in energy than its lowest energy state (perfectly crystalline), but there is a very large energy barrier between the two states. At room temperature such interstitial-vacancy clusters are stable for hours.

experiment, theory, and MD are emphasized. One now hopefully has a feeling for the variety and advantages of some potential types.

Also one should now be aware of some of the growing number of molecular dynamics acceleration methods – parallel-replica, temperature-accelerated dynamics, hyperdynamics, and on-the-fly Monte Carlo. Perhaps the most important information presented is the necessary background to understand what goes into an MD simulation. With this and the references, a motivated individual could probably code a simplistic MD simulator in a matter of a week (though given the increasing number of sophisticated, fast MD programs, writing one from scratch is probably not advisable except as a teaching tool).

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