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Defending Your DNA: Combating Threats both Foreign and Domestic

JAMES MCISAAC



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This paper originated as an in-class research assignment in Cell Biology, with Dr. Boriana Marintcheva. The subject matter is related to the research he conducts in the Biochemistry Research Lab. James plans to pursue his Ph.D. related to DNA damage tolerance and repair.

We are under constant assault from forces capable of damaging our DNA. The genetic code of DNA is made up of four nucleotides: adenine (A) which bonds with thymine (T) and guanine (G) which bonds with cytosine (C). If something happens to upset this normal pairing or the nucleotides themselves, our body must spring to action and respond to the damage. When damage to nucleotides prevents the normal replication machinery from doing its job, enzymes like the Y-family polymerases are called in. A special mechanism allows them to identify damage and insert the correct nucleotide or bypass the lesion to continue replicating a new strand of DNA. If not bypassed correctly, the result is a mutation in our genetic code that could lead to cancer. Understanding how our body reacts to genetic damage on a molecular level, will aid the development of future anti-cancer therapies.

Introduction:

Every day of your life there are soldiers that stand between you and certain danger. These soldiers are hidden from sight; waiting, watching, and always ready to protect you. They have abilities that set them apart from their peers and allow them to do the jobs that no one else could, but they do so with little to no oversight. What if, one day these soldiers act in a way to protect you, but something goes terribly wrong? What if these defenders make a mistake that puts your life in jeopardy? This is not the latest script for a Hollywood movie, but a scene that plays out every day inside every cell in your body. The stars of this real life action role are a group of enzymes commonly referred to as translesion polymerases. Every day, your DNA is under constant attack from enemies both foreign and domestic, or in biological terms, exogenous and endogenous, respectively. These attacks, when successful, often result in some type of damage. When DNA is copied, this damage, which is often referred to as a lesion, prevents the normal replication machinery from doing its job until the lesion is fixed by the DNA repair machinery of the cell. If the damage is too extensive, it then becomes the responsibility of the Special Forces enzymes that are able to perform a function called translesion synthesis (TLS). When these TLS polymerases step in and do what needs to be done to continue the DNA replication, errors in the code can be created. These errors are known as mutations and can be dangerous depending on which genes are affected. The mutations in DNA may translate into mutations in proteins, which may alter the way a protein functions or prevent it from working completely. The major focuses of this paper are the consequence of mutation,

types of damage to our DNA, and the enzymes responsible for coping with the damage.

Consequences of DNA damage:

The most well known consequence of mutation in DNA is cancer. In the 2012, it was estimated that an American was diagnosed with cancer every 19 seconds (1).

Long before a doctor can see evidence of a tumor, the process of cancerogenesis starts as a single genetic mutation. Our cells have an amazing array of defensive mechanisms to prevent these mutations from leading to cancer (2). Unfortunately, this becomes a numbers game in many cases. For 75 to 80% of cancer patients, their disease is the result of some external factor they came in contact with on a daily basis (3). The protective and repair mechanisms within our bodies can only cope with so much before failing or making a mistake. These genetic mutations can begin in the form of a DNA lesion, which is a structural anomaly in our genetic code caused by exposure to a residue or other harmful event capable of impacting our DNA. Our cells can either remove the lesion or attempt to replicate past it with special enzymes. If this damage is not properly handled a mutation may be present in future copies of the DNA. The impact a mutation will have on the body has more to do with how many are present, the gene possessing the mutation and what its normal function involves. The damage to our DNA comes in many sources and forms.

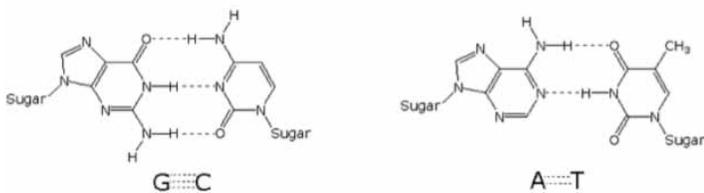


Figure 1 DNA nucleotides base-pairing. DNA is composed of four nucleotides whose order codes specific genetic messages in the double helix G (guanine) with C (cytosine) and A (adenine) with T (thymine). Each nucleotide is composed of a base (depicted with a chemical formula), sugar (depicted with the word sugar) and a Phosphate (omitted for clarity). The G-C and A-T bonding pattern determines the regularity of the DNA shape. Any mispairing or nucleotide chemical modification could cause a distortion of the double helix. This image was generated with ChemBioDraw Ultra.

Types of DNA Damage:

Our genetic code is determined by the sequence of four nucleotides: adenine (A), thymine (T), guanine (G), and cytosine (C) (Fig. 1). Under normal conditions each nucleotide only pairs with one other nucleotide. Adenine bonds with thymine and guanine bonds with cytosine. Each molecule bonds to its complementary pair in a specific way, creating the regular

shape of a double helix. Anything that disrupts this pairing or alters the shape of the nucleotides is known as a lesion. Some of the lesions found in DNA are abasic sites, interstrand & intrastrand crosslinks and adducts (8).

Abasic sites are lesions in which one member of a complementary pair of nucleotides is missing or twisted out of the DNA helix (7). These lesions can occur as the result of internal and external agents. There are several chemical interactions that could result in a nucleotide being excised from the DNA molecule. Enzymes could eject a nucleotide, or it could even happen spontaneously (7). When a nucleotide is missing or twisted out of alignment it causes a local and usually minor shift to the normal helical shape of a DNA molecule (7). This presents a two-fold problem for the normal replicative machinery. The normal enzymes must cope with the geometric distortion in the shape of the DNA molecule and the missing piece in the sequence to be copied.

Interstrand and intrastrand crosslinks are other types of lesions formed when nucleotides develop covalent bonds with another nucleotide instead of the normal pairing with its complementary nucleotide (6). When nucleotides on opposite strands form covalent bonds, they prevent the enzyme DNA helicase from performing its role of splitting the strands for replication (11). When these interstrand crosslinks or ICLs occur the cell will die if this damage is not repaired. Intrastrand crosslinks are formed when nucleotides on the same side form a covalent bond. These bonds can be caused by a variety of endogenous and exogenous agents. Thymine-thymine crosslinks are caused by exposure to ultra-violet radiation and are usually associated with different types of skin cancer (Fig 2). One way to remove this lesion is to eject one or both of the cross-linked nucleotides creating an abasic site (4). Alternatively, TLS polymerases can be used to bypass this lesion and allow replication to continue and prevent cell death (2).

Adducts are lesions in which DNA becomes covalently bonded to a molecule (5). These malicious chemicals can originate from a variety of sources. Given that there are so many potential chemical interactions that could occur, there are a tremendous number of possible adducts; a few examples are shown in Figure 3. Adducts distort the geometric shape of DNA to varying extremes, depending on the size of the adduct itself and where it occurs within the molecule (6). Normal replication enzymes are unable to synthesize new strands of DNA past these adducts, and enzymes like the Y-family of polymerases are called in to do the job (6). Etheno-dA is a lesion that can lead to mutation when cells try to copy DNA containing it (2). When hydroxyl-2-nonenal (or HNE for short) attacks a normal molecule of adenine, it forms a new bond that can

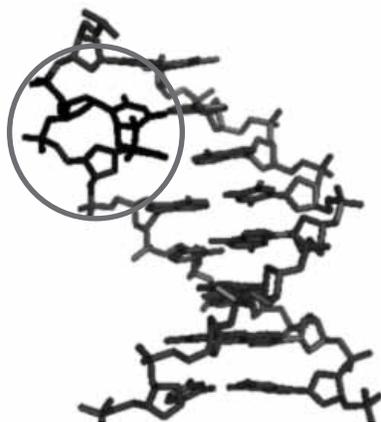


Figure 2 Distortion of a DNA by Thymine-Thymine Dimer. Double-stranded DNA adopts a regular helical shape driven by regular basepairing between complementary DNA bases. Thymine-Thymine dimers (circled in black) arise from crosslinking two adjacent Thymine bases upon UV irradiation and alter the overall shape of the DNA molecule. This image was generated by PyMOL. .

produce the etheno-dA lesion, altering the original structure in a way with which normal replication processes cannot cope (Fig. 3). The external influences that can generate these lesions are wide and varied, from the consumption of alcohol and coffee to cigarettes (9). Because of the change to the molecule of adenine, as shown in Figure 2 it is possible for the TLS polymerase to misread the nucleotide and incorporate a cytosine instead of a thymine leading to mutation (9). In order for cells to survive genetic damage they need special polymerases with their own set of rules.

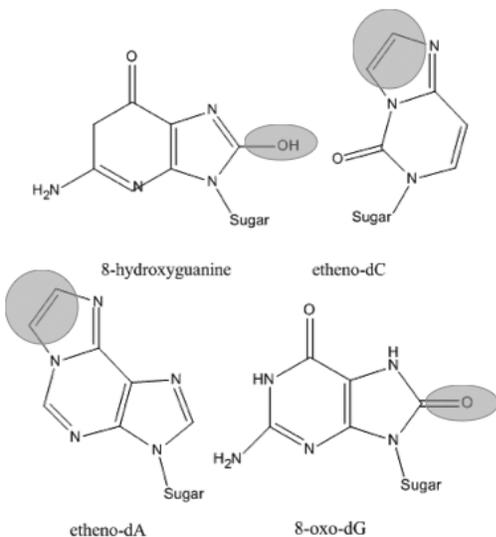
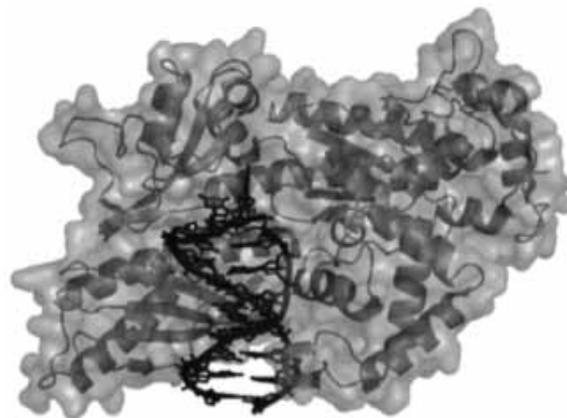


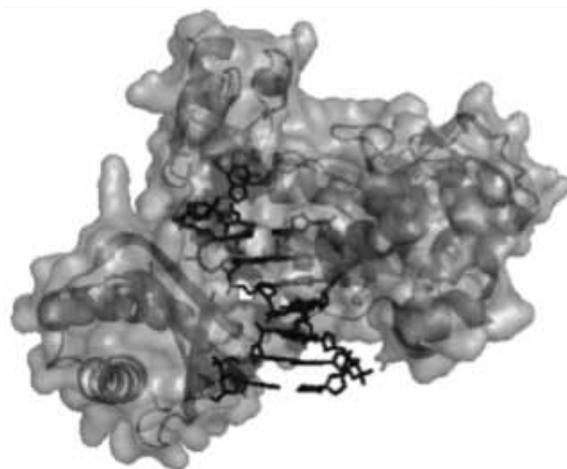
Figure 3 Examples of adducted nucleotides. Adducted nucleotides are chemically modified versions of Adenine, Thymine, Cytosine and Guanine. The chemical modification in each adduct is highlighted with a grey oval. The displayed chemical formulas were generated using ChemBioDraw Ultra.

Translesion Synthesis (TLS) polymerases:

In humans, translesion synthesis is largely attributed to five polymerases. Four of the polymerases are from the Y-family: **Kappa**, **Eta**, **Iota** and **Rev1**. Polymerase **Zeta** is from the B-family (6). Each polymerase has been shown to perform certain roles both individually and while working in tandem. The Y-family polymerases closely resemble each other, though with enough variation to perform different tasks (9). As with all proteins, function is directly related to structure. While these enzymes perform related tasks, the lesions they bypass are structurally different and require their respective bypass enzymes to have sufficient variation in their active sites to accommodate these lesions (Fig 4). These enzymes have been described as resembling an open right hand, and the regions of this enzyme have been labeled according to that analogy (Fig 5). They have a thumb that holds the DNA in a region called the minor groove. The polymerase finger(s) lie over the template nucleotide to be copied. The palm creates a bond with the phosphate backbone of the DNA molecule. There is a section of the enzyme called the N-digit or little finger which has some variation in functionality between the enzymes. The wrist or polymerase associated domain (PAD) contacts the ma-



A



B

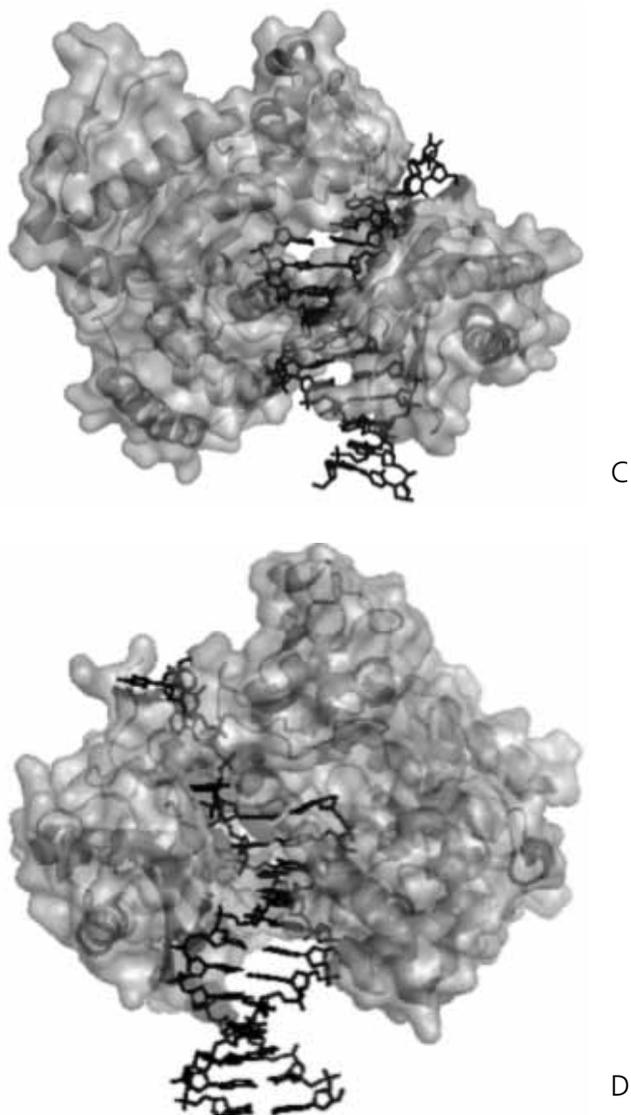


Figure 4 Y-Family DNA polymerases. A) Pol. Eta (PDB 2R8J) B) Pol. Iota (PDB 3GV8) C) Pol. Kappa (PDB 3IN5) D) Rev1(PDB 2AQ4). Each polymerase is visualized interacting with DNA to give a perspective on the size of each enzyme and its active site with respect to the other enzymes in the family. These four polymerases are structurally different as seen by the above images. The variation in structure allows for lesions of various sizes to fit within the active site of the appropriate enzyme. The image was generated using PyMOL Molecular Graphics System using crystal structures obtained from the PDB.

gor groove region on DNA (9). Some obstructions prevent a single enzyme from both bypassing a lesion and restarting the replication process for the rest of the DNA strand to be copied; and therefore must work with a partner (9). If TLS is done correctly, it allows DNA to replicate, despite the initial presence of an anomaly, though this replication is not always error free. The error rate for a TLS polymerase is between 10^{-2} and

10^{-4} , while normal replication polymerases have an error rate between 10^{-5} and 10^{-7} (3). It is important to note that because TLS polymerases do not have any proofreading component, like polymerases involved in normal replication, this can result in mutations that are not corrected.

Pol Eta is capable of working independently or in conjunction with one of its sibling enzymes. This enzyme has proven efficient at dealing with lesions caused by ultra-violet rays (9). Cyclobutane pyrimidine dimers or CPDs are lesions caused by UVB and UVC rays, and Pol eta is the most reliable at bypassing these lesions without mutation (9). It can also perform translesion synthesis past some types of intercross links or ICLs. Bulky adducts like 8-oxoguanine are bypassed by this polymerase also. The thymine-thymine crosslink, like that shown in figure 2 which can lead to skin cancer, is bypassed by this enzyme along with Rev1 (4). Pol eta is able to insert two adenines across from the two thymines, but it requires Rev1 to restart the replication process (4).

Pol Iota is considered the highest fidelity, or most faithful polymerase in the Y-family (9). Pol iota is also capable of bypassing thymine-thymine crosslinks though not as well as Pol eta (9). Incorporation of the correct nucleotide is more efficient with adenine and guanine as the template than cytosine and thymine (9). It has been shown that abasic sites can be bypassed by Pol iota with good efficiency. As well as certain guanine adducts, and a wide variety of adenine adducts. Though capable of bypassing several lesions, it is often inefficient at extending replication past the lesion and must work with a partner to perform both functions (9).

Pol Kappa is considered to be fairly high fidelity for a Y-family polymerase. It has been shown to work with other polymerase enzymes, where it takes on the role of extending replication past the lesion site (9). It has proven effective for certain types of ICS bypass (4). Pol kappa is well known for being able to bypass bulky guanine adducts like Benzo(a)pyrene diolepoxide (BPDE), and adduct associated with cancer caused by cigarette smoke (2). It will also work with other enzymes like Rev1 when bypassing adducts like thymine glycol, where Rev1 is responsible for restarting the replication process past the lesion (2). Pol kappa has also been shown to both bypass abasic sites and cause them (2). When it causes an abasic site by removing a nucleotide it often causes another problem known as a frame-shift error or mutation (9). These frame-shift errors are often the result of an adjustment caused by a misinsertion of a nucleotide. The enzyme tries to force out the incorrect sequence and reinsert it across from the next appropriate nucleotide (9). This can cause slight distortion to the frame of the DNA helix even after replication has restarted.

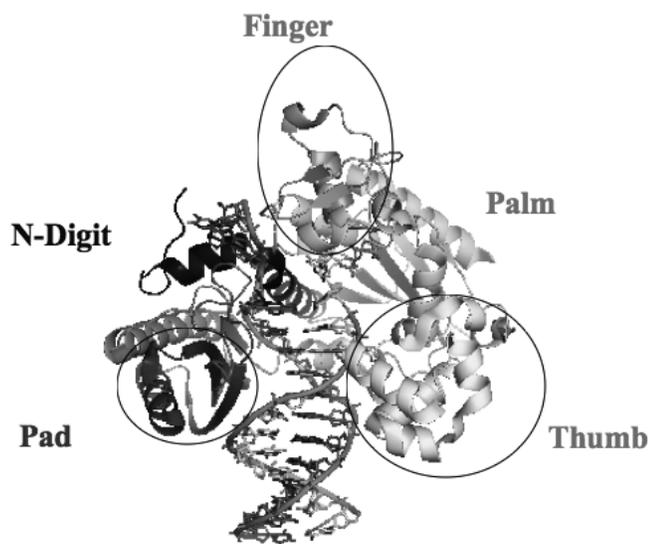


Figure 4 Rev1 Molecular Structure. DNA polymerases 3D structure is often described as an analogy of a Right Hand composed from Thumb, Palm, Fingers, PAD and N-digit as depicted above. The characteristics of the Palm, PAD, Fingers, Thumb and N-Digit vary slightly between the polymerases allowing them to perform their specific tasks. While there is variation, the basic configuration is the same throughout the family. This figure was generated using PyMOL and PDB 2AQ4 molecular coordinates.

The complete role of **Rev1** is still being uncovered. Rev-1 typically binds to a guanine and inserts a cytosine when reading and copying DNA (11). Although Rev1 prefers inserting a cytosine opposite a guanine, it will bind to other sites, although much less efficiency is observed (6). When Rev1 interacts with the DNA helix, the amino acid glycine forms specific interactions on guanine (6). Once bonded to the guanine, Rev1 is then able to insert a cytosine into the new strand of DNA to allow proper coding to continue. If Rev1 does not do its job in this fashion, the new strand will contain a potentially hazardous mutation. Bypassing guanine adducts is what this polymerase is best known for, which is a poor choice because it focuses on one aspect of what this enzyme is capable of. Rev1 has been shown to possess the unique ability of interacting with the rest of the TLS polymerases. The N-digit on Rev1 allows it to interact with other enzymes and accessory proteins (4). With these protein interactions Rev1 makes it possible for lesion bypass to occur with the continuation of the replication process without stalling and putting the DNA strand in more jeopardy.

It is nearly impossible not to talk about **Pol Zeta** when speaking on the subject of translesion synthesis. The other two enzymes in the B-family have a proofreading functionality that, like the Y-family, Pol Zeta lacks. While it may lack proofreading, it is still a higher fidelity polymerase than most of the Y-family (5). It has been experimentally determined that Pol zeta can work

with other enzymes to allow replication to continue past the site of a lesion, or a bypassed lesion. Pol Zeta is structurally different from the Y-family, as it is composed of only two subunits called Rev3 and Rev7 (4). Pol zeta in conjunction with Rev1 is blamed for many of the mutations found in eukaryotes (12). These two enzymes together are able to bypass many lesions and incorporate the wrong nucleotide.

Looking to the future:

According to Sun Tzu, “the highest form of generalship is to balk the enemy’s plans, the next best is to prevent the junction of the enemy’s forces, the next in order is to attack the enemy’s army in the field, and the worst policy of all is to besiege walled cities” (13). Fighting a walled city is exactly what we are doing when we attack cancer that has already formed a tumor. This city has an ever-increasing population, and its own blood vessels supply it with all of the resources it needs to fight back. We are often forced to fight cancer with weapons that attack the entire body, not just the tumor. As we are exposed to chemotherapy drugs, they create the risk of developing new cancerous mutations. In no way is this a wise strategy for winning a war. With more research aimed at understanding the mechanics behind a cell becoming cancerous, we can switch our tactics and focus on balking the enemy’s plans.

Cancer is an enemy that does not confine its damage to the person it attacks, but unleashes its malicious influence in some way upon everyone in the victim’s life. During 2010, American families spent an estimated \$124.57 billion dollars on cancer care (1). This enormous financial burden is the result of doctor visits, clinic treatments, procedures, lab tests, imaging tests, radiation treatment, drug costs, hospital stays, surgeries, and home care (1). The financial breakdown was a lot to fit into a sentence; imagine fitting it into your life. What if there was another way to fight cancer? Without a better understanding of this process we will never learn to ask better questions. As more research is done to understand the role each enzyme plays during lesion bypass, the closer we come to developing targeted treatments and more comprehensive preventative measures.

Works Cited

1. American Cancer Society. *Cancer Facts & Figures 2012*. Atlanta: American Cancer Society; 2012
2. Caixia, Guo, et al. “Mouse Rev1 protein interacts with multiple DNA polymerases involved in translesion DNA synthesis”. *The EMBO Journal*. 22. 24. (2003). 6621-6630.
3. De Bont, Rinne, Van Larebeke, Nik. “Endogenous DNA damage in humans: a review of quantitative data”. *Mutagenesis*. 19.3. (2004): 169-185

4. Deepak Nair, et al. "Rev1 employs a novel mechanism of DNA synthesis using a protein template". *Science*. 309. 2219. (2005). 2219-2222.
5. Frank, Alexander et al. "Immunohistochemical detection of 1,N⁶-ethenoxyadenosine in nuclei of human liver affected by diseases predisposing to hepato-carcinogenesis". *Carcinogenesis*. 25. 6. (2004). 1027-1031
6. Janice D. Pata, et al. "Structural diversity of the Y-family DNA polymerases". *Biochimica et Biophysica Acta*. 1804. (2010). 1124-1135.
7. Jeong-Yun Choi, et al. "Translesion synthesis across abasic lesion by human B-family and Y-Family DNA polymerases". *Journal of Molecular Biology*. 404. (2010). 34-44.
8. Rinne De Bont, et al. "Endogenous DNA damage in humans: a review of quantitative data". *Mutagenesis*. 19. 3. (2004). 169-185.
9. Satya Prakash, et al. "Eukaryotic translesion synthesis DNA polymerases: Specificity of structure and function". *Annu. Re. Biochem*. 74. (2005). 317-353.
10. Swan, Michael et al. "Structure of human Rev1-DNA-dNTP ternary complex". *Journal of Molecular Biology*. 390. 4. (2009). 699-709.
11. Vinh Ho, et al. "Structure-dependent bypass of DNA interstrand crosslinks by translesion synthesis polymerases". *Nucleic Acids Research*. 39. 17. (2011). 7455-7464.
12. Ying Zhou, et al. "The catalytic function of the Rev1 dCMP transferase is required in a lesion-specific manner for translesion synthesis and base damage-induced mutagenesis". *Nucleic Acids Research*. 38. 15. (2010). 5036-5046.
13. Sun Tzu. *The Art of War*. Trans. Samuel B. Griffith. New York: Oxford University Press, 1971. Print.