Can latency-reversing drugs reawaken dormant retrovirus infection? A mathematical analysis

S. Chen (chen@rheast.com)
Rheast LLC

Research Article

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Can latency-reversing drugs reawaken dormant retrovirus infection? A mathematical analysis

S. Chen
Rheast LLC, 1331 Lamar St, Houston, TX 77010, USA. chen@rheast.com

Background:
The technique of using drugs to target latent virus reservoir has been introduced to reawaken the dormant virus so that the immune system can attack it. However, further tests have shown this method to fail in laboratory tests. In this work, the author tries to mathematically analyze whether drugs can be used to reawaken dormant virus reservoirs. The study uses mathematical formulas to differentiate between different relationship types, and then a model of protein reawakening dormant reservoirs is presented.

Results:
The results show that the amino acid sequences ARG of gag proteins of HTLV1, HTLV2, STLV1 and STLV2 match with their primer binding site GGGGGCTCG in the 3'-to-5' direction, and the amino acid sequences SPR of gag proteins of HIV1, HIV2, SIV and FIV match with their primer binding site GGCGCCCGA in the 3'-to-5' direction. The gag, gag-pol and gag-pro-pol proteins are promising for reawakening dormant retrovirus infection.

Conclusions:
The author hence believes that the latency-reversing drugs were involved in the process of transcription of cancer, and the genome they reawakened just happened to contain the genome of the retrovirus, which means that it was false reawakening. On the other hand, using proteins of retroviruses to reawaken them is more reliable, just like androgen receptor activates the *IGF1R* gene.

Keywords:
Retrovirus, HIV, HTLV

1. Background

The acquired immunodeficiency syndrome (AIDS) is a disease caused by the human immunodeficiency virus (HIV). The virus attacks immune system cells in the body to use their machinery to make copies of itself. However, some HIV-infected immune cells enter a state in which they do not produce new virus, called the resting or latent state. These form latent HIV reservoir, in which HIV can hide for years, avoiding HIV therapy. At any time, these cells can become active again and start to make more copies of the virus [1]. Scientists have used this opportunity to develop methods to target these latent reservoirs and make them active, so that they can be identified and targeted by HIV therapy. However, scientists at Johns Hopkins reported that compounds they hoped would 'wake up' dormant reservoirs of HIV inside the immune system. T cells have failed to do so in laboratory tests on white blood cells taken directly

from patients infected with HIV [2]. Hence more investigation is needed to decide on the applicability of this method. In this article, the author tries to mathematically analyze whether latency-reversing drugs can reawaken the sleeping retrovirus.

2. Methods

2.1. Differentiating between the contain and equal relationships

The study designed an experiment as follows. First, the author prepares several T cells and the HIV1 double-stranded DNA, which are converted by reverse transcription. HIV genome contains at least nine genes, such as gag, pol and env [3]. The *IGF1R* gene is located on the human chromosome 15, which contains at least 21 exons, such as ENSE00003838363 and ENSE00001316091 [4]. In mathematics, a set is a collection of elements, so the genome can be defined as a set of elements, by listing its elements between curly brackets, separated by commas:

$$H = \{\text{gag}, \text{pol}, \text{env}\} \qquad I = \{\text{ENSE00003838363}, \text{ENSE00001316091}\ \}$$

Where H denotes the set of HIV genome, and I represent the set of the *IGF1R* gene. Next, the CRISPR-Cas9 enzyme [5,6] is used to copy the HIV genome into the *IGF1R* gene of T cells. The set can be rewritten to:

$$I = \{\text{ENSE00003838363}, \text{gag}, \text{pol}, \text{env}, \text{ENSE00001316091}\ \}$$

The *IGF1R* gene is one of the known target genes of androgen receptor activation [7]. In mathematics, a function from a set X to a set Y is an assignment of an element of Y to each element of X. Hence, the process of transcription can be written in the following form:

$$f(x) = y \qquad f(A) = I \qquad A = \{\text{androgen}, \text{androgen receptor}\}$$

Where f is the function of RNA polymerase II, and A denotes the collection of androgen and its receptor. Then, the androgen and its receptor are injected into the T cells. After the *IGF1R* gene is transcribed by RNA polymerase II [8], the HIV will also 'wake up' [9]. Python is one of the most popular programming languages [10], which can be used to write scripts to check the accuracy of mathematical formulas:

```
#!/usr/bin/python3
H = {'gag','pol','env'}
I = {'ENSE00003838363','ENSE00001316091'}
I.update(H)
```

```
A = {'androgen','androgen receptor'}
def f(x):
    if x == A:
        return I
```

The set H is defined to represent the HIV genome and the set I represents the *IGF1R* gene, then the update method is used to insert H into I. Next, a set A is defined to represent androgen and its receptor, while $f(x)$ is defined to represent the function of RNA polymerase II, which returns the *IGF1R* gene applied

on the set A. At last, print $H \leq f(A)$ to verify whether the virus is activated or not. As a result, the Python program returns True, which indicates that the dormant HIV infection is reawakened.

```
9 | print(H <= f(A)) #True
```

Can this possibly mean that the androgen reawakens the sleeping HIV? The answer is that the androgen reawakens the *IGF1R* gene that contains the HIV genome, not the retrovirus directly. In fact, even the Python program returns a result of False.

```
10 | print(H == f(A)) #False
```

It can be seen that the collection of elements returned by the method includes the HIV set, which doesn't mean that the two sets are equal. In mathematics, their relationship can be expressed as follows:

$$H \subseteq f(A) \qquad\qquad H \neq f(A)$$

Several NIH-funded studies claim that AZD5582 can reawaken the sleeping HIV and SIV, but they also claim that the effectiveness rate is only at 42% [11,12,13]. Most importantly, the novel small-molecule IAP inhibitor AZD5582 was used for the treatment of cancer. It was reported to cause cIAP1 degradation, inducing apoptosis in the MDA-MB-231 breast cancer cell line at subnanomolar concentrations in vitro [14]. AZD5582 was involved in the process of transcription of cancer genes, and the genome it actually reawakens just happened to contain HIV genome, so it is a false reawakening.

2.2. Differentiating between the indirect and direct activation

Besides AZD5582, many studies claim that latency-reversing drugs can be used to reawaken the sleeping HIV, including Ciapavir [15], bryostatin-1 [16], disulfiram [17], ingenol-B [18], prostratin [19].

What could be the mechanism behind this? In molecular biology, a base pair is a fundamental unit of double-stranded nucleic acids consisting of two nucleobases bound to each other by hydrogen bonds, such as adenine-thymine (AT) and guanine-cytosine (GC) [20]. Hydrogen bonds are usually formed between atoms that are electronically complementary, i.e., between a proton acceptor atom with a partial negative charge and an opposing proton atom with a partial positive charge [21], as shown in Fig. 1.

Figure 1. Watson-Crick base pair

Therefore, in order to transfer the electromagnetic interaction [22], the latency-reversing drugs should

also be matched with the corresponding nucleobases. Unfortunately, the atoms that constitute these drugs are completely different. Even if their atoms are all matched with corresponding nucleobases, e.g., an oxygen atom and a hydrogen atom as thymine, the formed sequences are still different and cannot match with the same genome of the same virus at the same time. In fact, disulfiram doesn't even have an oxygen atom, as shown in Fig. 2.

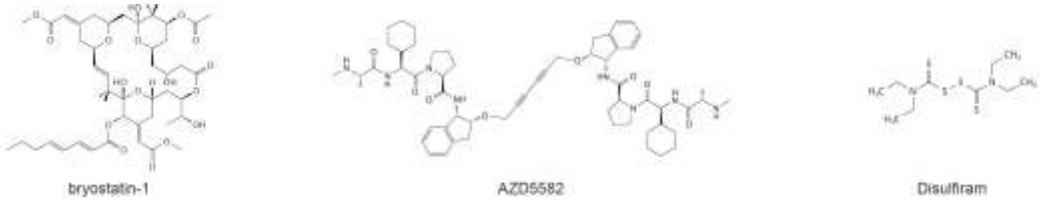

Figure 2. Latency-reversing drugs

More importantly, the author seeks to define which atom is the starting point and which is the ending point. Furthermore, the author tries to know how does RNA polymerase II read each atom and in what order. Since these drugs do not have regular patterns of structure, they cannot be directly identified by the RNA polymerase II. On the other hand, the one that has a fixed structure can be identified by the polymerase, e.g., the androgen receptor, which has amino acid sequences that can be sequentially read in a fixed order.

In addition, these drugs have completely different shapes and sizes, and if two atoms are far away from each other, then the electromagnetic interaction is negligible. The electromagnetic force that occurs between two electrically charged particles can be mathematically expressed according to Coulomb's law [23] as follows:

$$F = k_e \frac{q_1 q_2}{r^2} \qquad k_e = \frac{1}{4\pi\varepsilon_0}$$

Where k_e is the Coulomb's constant, q denotes the signed magnitudes of the charge, r represents the distance between two charges, and ε_0 is the vacuum permittivity. It can be seen that when the distance is more than 1 nm, the electromagnetic force between two atoms will be less than 1% of the force at the distance of 0.1 nm. The androgen receptor, which is made up of hundreds of proteins, is much bigger than the latency-reversing drugs.

If the connection at the molecular level is magnified to the macro-level, it would be as if those scientists are trying to put different types of plugs into one completely mismatched receptacle. An example is illustrated in Fig. 3.

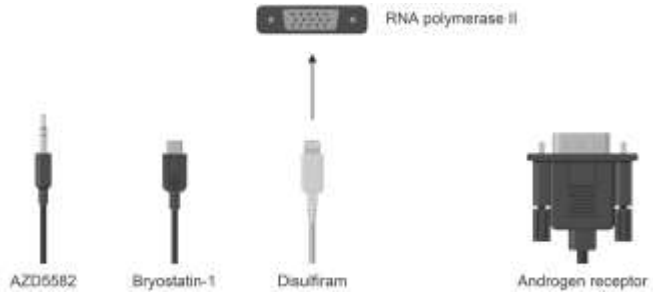

Figure 3. Drugs reawaken

In computer science, in order to make one receptacle match with different plugs, each of these plugs requires a unique adapter to convert the electromagnetic interaction. Similarly, the androgen is adapted by its receptor, and it is the androgen receptor that directly activates the *IGF1R* gene, not the androgen.

While these conclusions do not doubt the NIH-funded studies, the blame is on the RNA polymerase II. The one that made the mistake must be the polymerase, which has countless protein structures in a superposition and can automatically match with every latency-reversing drug, accurately converting the electromagnetic interaction without the receptors. It is worth mentioning that those latency-reversing drugs were also used for the treatment of cancer [24,25,26,27], just like AZD5582 [14].

2.3. The proposed model

It is well known that HIV recruits human uncharged tRNA to serve as the reverse transcription primer [28]. The HIV genome and *IGF1R* gene are both transcribed by RNA polymerase II [8,9]. The *IGF1R* gene is one of the known target genes of androgen receptor activation [7], and the androgen receptor is recruited by RNA polymerase II [29]. A promoter is a DNA sequence that leads RNA polymerase II to the correct initiation site [30]. Adenine, cytosine and guanine are found in both RNA and DNA, uracil and thymine are both bound to adenine via two hydrogen bonds [31], and tRNA serves as the physical link between the mRNA and the amino acid sequences of proteins [32].

Therefore, the author proposed a model of protein reawakening includes that uncharged tRNA serves as the physical link between the promoter and the protein receptors, which is recruited by RNA polymerase II. An example is illustrated in Fig. 4 [33,34,35].

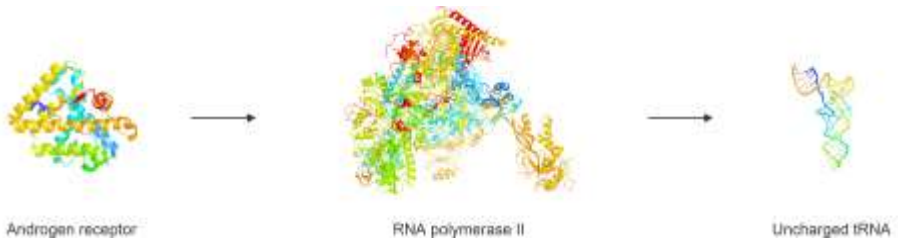

Figure 4. Protein reawakens

Since retroviruses can accurately identify themselves at any time, either in the process of reverse transcription or assembling themselves, which means the thing that has been used to identify themselves can be constantly replicated all the time. So, what can actually reawaken themselves is their own proteins, for example, the gag, gag-pol and gag-pro-pol proteins. To determine which amino acid sequences matched their primer binding site, a Python program was written to match all the proteins with their own gene sequences and display them graphically.

3. Results

DNA is always synthesized in the 5'-to-3' direction, but reverse transcriptase synthesizes negative-strand DNA in the 3'-to-5' direction [36,37]. The androgen receptor recruited by RNA polymerase II may also be rotated 180 degrees, which creates 4 ways for uncharged tRNA to match the primer binding site. The author uses the x-axis to represent the protein, and the y-axis to represent the primer binding site. Negative numbers indicate that the protein or tRNA rotated 180 degrees or was synthesized in the 3'-to-5' direction when both values were negative.

Having 2 amino acid sequences of the matching points leads to many possibilities, such that it is impossible to confirm which protein matches the primer binding site. When there are 4 amino acid sequences, no matching target can be found. However, when there are 3 amino acid sequences, there is exactly one perfect matching region. Different types of retroviruses are represented with different patterns and colors, and their sequences around the primer binding site are matched with their own proteins. as shown in Fig. 5.

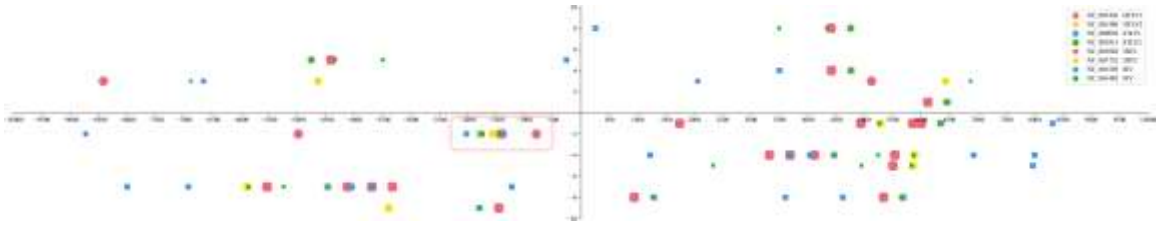

Figure 5. Coordinates of matched points

Fig. 5 shows that inside the red box, the coordinates of 8 different viruses appear at the same time, and they are extremely close, which means they represent the same protein. In other locations, there was either only Deltaretrovirus or only Lentivirus, and the spacing between the different color coordinates was too large, indicating that they were not even the same protein and were therefore excluded.

If no protein can be matched with the primer binding site on the same y-axis, the fragment of the primer binding site will not be matched with the uncharged tRNA, so the same y-axis of the same type of virus can be removed to reduce the data interference. The virus amino acid sequences of the protein mutated, while its primer binding site remained the same, which means that it was not the matching target. More importantly, the proteins that can be matched with the same primer binding site must belong to the same type, which has the same function and participates in the process of reverse transcription.

In the GenBank database, the primer binding site of the HTLV2 NC_001488 genome is around 766 to 783, and that of the HIV1 NC_001802 genome is around 182 to 199. It can be seen their primer binding sites start with TGG and end with GGGA, and after aligning the sequences, their matching points can be found in the same position, as shown in Fig. 6 [38,39,40,41,42,43,44,45].

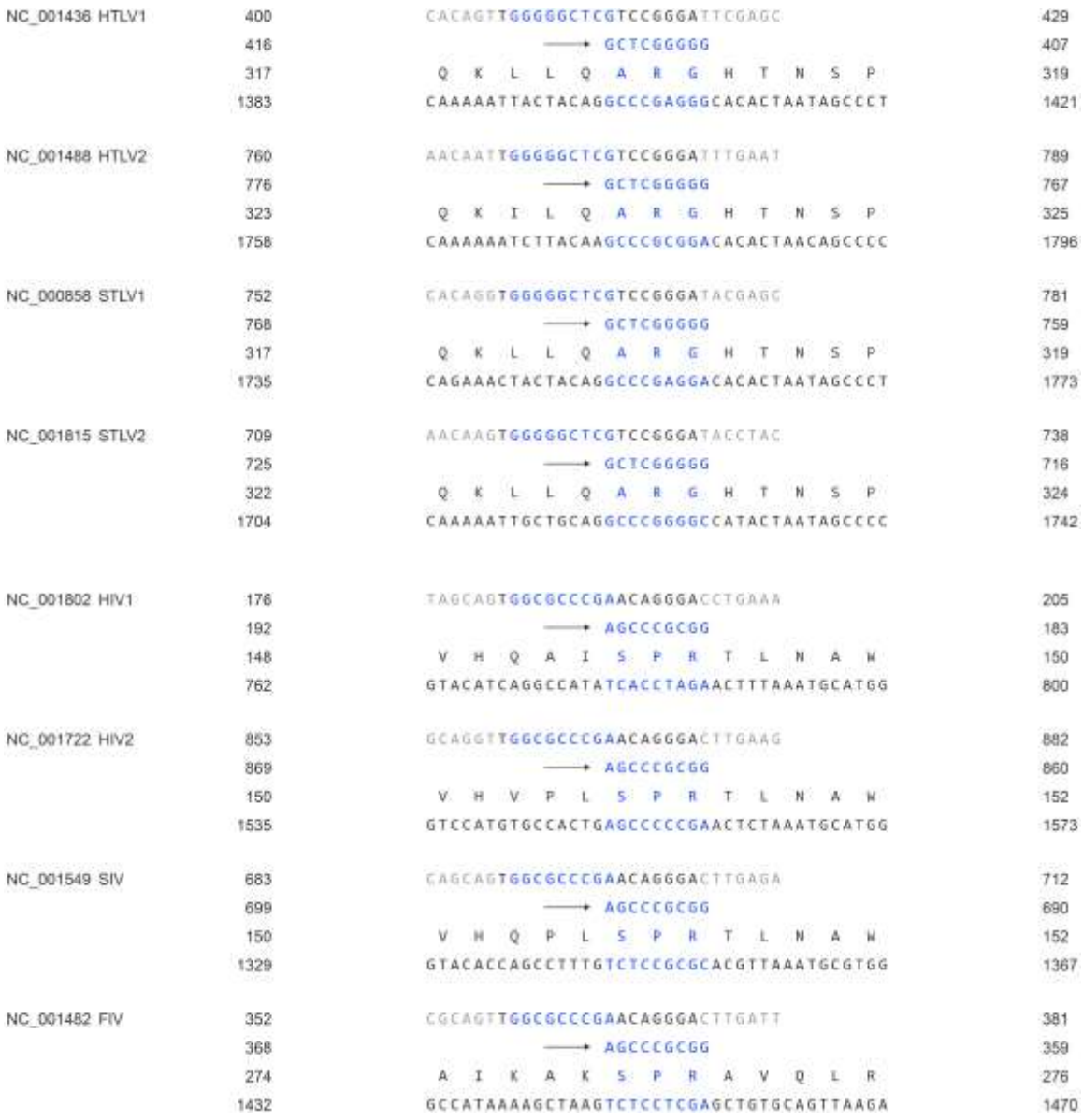

Figure 6. Deltaretrovirus and Lentivirus

So, it can be determined that their gag, gag-pol and gag-pro-pol proteins match with the same primer binding site, even though the viruses are highly different.

4. Discussion

It is possible that other proteins were also involved in this process, but there is no sufficient information to determine this now. What can certainly be concluded is that so far, drugs fail to reawaken dormant HIV infection, and it is more reliable to use proteins of retroviruses to reawaken them, just like the androgen receptor activates the *IGF1R* gene. With the latency-reversing drugs failing for the last 40 years, this study can speculate that they will keep failing in the next 40 years because drugs cannot reawaken dormant retrovirus infection without the corresponding protein receptor.

5. Conclusions

The amino acid sequences ARG of gag proteins of HTLV1, HTLV2, STLV1 and STLV2 match with their primer binding site GGGGGCTCG in the 3'-to-5' direction, and the amino acid sequences SPR of gag proteins of HIV1, HIV2, SIV and FIV match with their primer binding site GGCGCCCGA in the 3'-to-5' direction. The gag, gag-pol and gag-pro-pol proteins are promising for reawakening dormant retrovirus infection.

List of abbreviations

IGF1R: Insulin-like growth factor 1 receptor
HTLV: Human T-lymphotropic virus
STLV: Simian T-lymphotropic virus
HIV: Human immunodeficiency virus
SIV: Simian immunodeficiency virus
FIV: Feline immunodeficiency virus

Ethics approval and consent to participate

Not applicable.

Consent to publish

The author gives the consent for the publication of identifiable details, which can include photographs and details within the text to be published in the above Journal and Article.

Availability of data and materials

Datasets were produced by Python3, tool available at https://github.com/rheast/genome. Nucleotides were downloaded from NCBI database https://www.ncbi.nlm.nih.gov/nuccore/. Samples nucleotides correspond to accession numbers: NC_001436, NC_001488, NC_000858, NC_001815, NC_001802, NC_001722, NC_001549 and NC_001482.

Competing interests

There are no conflicts of interest.

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None.

Authors' Contributions
S.C. wrote the manuscript.

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None.

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