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Chemosensitising Non-Hodgkin Lymphoma cells with protein synthesis inhibitors

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Abstract

Non-Hodgkin lymphomas (NHL) accounts for 2.6% of all cancer deaths in England and Wales and the incidence is increasing every year. Experimental data suggest that in NHL cells there are changes that would lead to an upregulation of anti-apoptotic proteins, down regulation of pro-apoptotic signals, and upregulation of proteins involved in cell adhesion. Chemicals that inhibit translational initiation include inhibitors of p38Mapkinase pathways (e.g. SB203580), ERK pathway (e.g. PD98059) and FRAP/mTOR pathway (e.g. Rapamycin and Wortmanin). We present a mathematical model of the signalling pathways associated with capped translation and use the model to predict whether it is possible to use translational inhibitors to chemosensitise NHL cells. We wish to determine whether it is possible to alter the expression of the anti-/pro-apoptotic mRNAs by treating NHL cell lines with these inhibitors.

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1 Motivation

1.1 Biological Background

B cells and T cells are the major types of lymphocytes. Non-Hodgkin lymphomas (NHL) comprise at least thirty distinct subtypes of B and T cell malignancies although over 90% of NHL are of B cell origin. At present NHL account for 2.6% of all cancer deaths in England and Wales and the incidence is increasing by approximately 5% per year, although reasons for this increase are unknown.

DNA can only exert an effect on its cell when transcribed into mRNA (in the nucleus) and subsequently translated from mRNA into proteins (outisde the nucleus)[1]. The control of transcription and translation is therefore fundamental to any cell's function.

In eukaryotes, translation of mRNA into proteins is carried out by a variety of protein complexes, which themselves were created by translation[2]. There tend to be two main mechanisms used, which can be differentiated by whether or not they require a certain 'cap' protein — these are called cap-dependent (capped) and cap-independent (uncapped) translation. Capped translation favours the production of proteins involved in growth and proliferation, whereas uncapped translation favours the production of proteins involved in apoptosis (programmed cell-death). By favouring one form of translation over the other the cell 'chooses' whether to live or die.

mRNAs are directional strings of nucleic acids, designed to be read in one direction only (termed 5' to 3'). At the beginning there is a region which is not translated into protein (a 5' un-translated region or 5'UTR), which is thought to act to help translation machinery attach. The translation machinery is made up of eukaryotic translation Initiation Factor proteins (eIFs), which allow ribosomal subunits (made up of RNA) to bind to mRNA. Assisted by eIFs the ribosomal subunits form ribosomes. Once attached the ribosomes work their way along the mRNA producing a string of amino acids, which fold into a protein. mRNAs with a highly structured 5'UTR appear to code for more proteins involved in cell proliferation and growth than proteins involved in apoptosis; it is these structured mRNAs which are favoured for capped translation.

In healthy cells under high growth factor signals around 90% of translation is capped[1] (more 'life' than 'death' proteins are expressed) and the cell lives, grows and proliferates. If the external growth factors are removed then the amount of capped translation can drop below the level of uncapped translation (more 'death' than 'life' proteins are expressed) and the cell may undergo apoptosis and die. This is the main mechanism for controlling whether or not a cell should undergo apoptosis[2].

NHL cells have acquired mutations which cause changes in the concentrations of proteins (such as ERK, mTOR and the eIFs) which influence the rate of capped translation[4]. This leads to up-regulation of capped translation under both high and low growth factor conditions, such that the cells never have a higher level of uncapped translation, and therefore do not die when 'instructed to do so' by outside signals. This causes hyper-proliferation and leads to the initiation of cancerous tumour formation.

Recent experiments have given us quantitative measurements of the relative change in concentration of these proteins between NHL and healthy cells.

1.2 The Experimental Data

The data suggest that in NHL cells there are changes that would lead to an upregulation of global rates of protein synthesis alongside up-regulation of anti-apoptotic proteins (e.g. DAXX) and down regulation of pro-apoptotic signals (e.g. TNF) and upregulation of proteins involved in cell adhesion (e.g. LAMB2). In Table 1 we display some of the results, where proteins in the pathway governing capped translation are present at different concentrations in NHL and healthy cells.

[1] The exception being genes which code for cell machinery made from RNA, such as rRNA and tRNA, which are never translated.
[2] This is one reason why DNA can never be the sole unit of inheritence[3].

Protein	Parameter	Healthy Cells	NHL Cells
Grb2	M_T	1	1.4
AKT	A_T	1	1.33
mTOR	T_T	1	0.69
e1f4B	F_T	0.5	2

Table 1: The ratio between up/down-regulation of proteins in NHL cells relative to healthy cells. These results come from polysomal profiling in the Willis lab. Here we have suggested their effect on the parameters we introduce in section 2.

1.3 The Study Group Questions

Chemicals that inhibit translational initiation include inhibitors of p38Mapkinase pathways (e.g. SB203580), ERK pathway (e.g. PD98059) and FRAP/mTOR pathway (e.g. Rapamycin and Wortmanin). We wish to determine whether it is possible to alter the translation levels of anti/pro-apoptotic mRNAs by treating the 6 NHL cell lines with these inhibitors. We wish also to establish whether this pre-treatment would sensitise cells to the effects of chemotherapeutic agents.

The Study Group is asked to develop mathematical models of the signalling pathways associated with NHL and to use these models to predict whether it is possible to use translational inhibitors to chemosensitise NHL cells.

2 Modelling

In this section we describe the signalling pathways and complex formations that we will model, before writing down equations to describe them.

2.1 Protein Interactions

We will consider control of capped translation in two parts. Firstly, signalling pathways mediate the external growth factor signals received by the cell, and secondly in response a complex forms around the capped end of mRNAs, resulting in binding and activation of ribosomes (the translation machinery).

The signalling pathways

Two signalling pathways control the assembly of the capped translation complex. The first pathway we will call the mTOR pathway, the second the ERK pathway. Both pathways have some common stimuli which we will call simply a growth factor.

In the mTOR pathway (see right hand side of Figure 1) the growth factor causes the upregulation of PI3K (a target of the inhibitory drug Wortmannin), this leads to the upregulation of AKT, which in turn leads to the upregulation of mTOR (a target of the inhibitory drug Rapamycin). In the ERK pathway (see left hand side of Figure 1) the growth factor causes the upregulation of Grb2, which leads to an increase in MEK, which in turn leads to upregulation of ERK (a target of inhibitory drugs such as PD98059).

Capped translation initiation

Figure 2 shows the formation of the capped translation machinery: the 'cap' protein (e1F4B) is usually sequestered by another protein (4E-BP). Under the stimulation of mTOR (T^*), 4E-BP is phosphorylated and

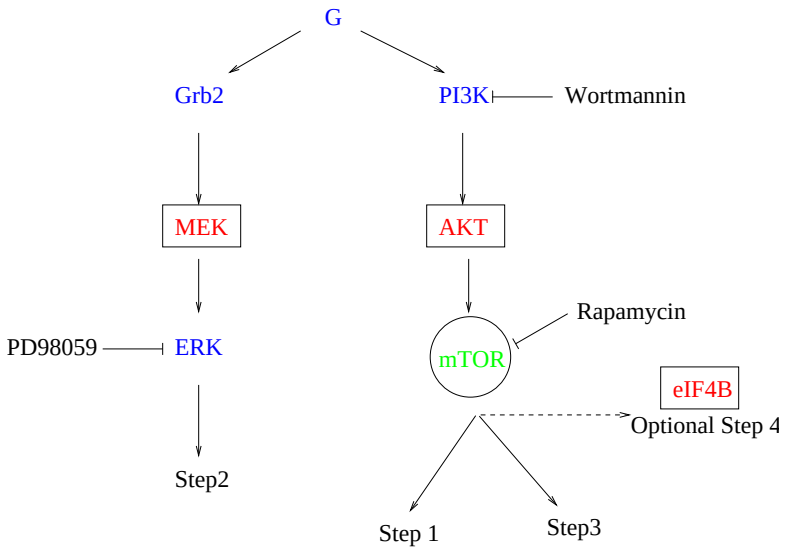

Figure 1: Signalling Pathway: in the mTOR pathway the growth factor causes the upregulation of PI3K, this leads to the upregulation of AKT, which in turn leads to the upregulation of mTOR . In the ERK pathway the growth factor causes the upregulation of Grb2, which leads to an increase in MEK, which in turn leads to upregulation of ERK. The 'steps' at the bottom of the figure relate to promotion of the steps in capped translation complex formation shown in Figure 2. Key: squared proteins are up-regulated and circled ones are downregulated in NHL cells (see Table 1).

releases eIF4B.

A scaffold protein C_0 exists in a free state in the cytoplasm, it can bind to free e1F4B to form a complex we have denoted C_1. mRNAs bind to C_1 to form a circle, their 5' cap ends bind to e1F4B, whist the 3' polyA tail binds to part of the scaffold protein; this process is facilitated by ERK (E^*) and we denote the resulting complex (scaffold, e1F4B and an mRNA) as C_2. The circular structure enables ribosomes to translate an entire protein whilst returning to the 'start' to repeat the process. Under the action of mTOR a ribosomal subunit S6 joins C_2 to form a complex we denote by C_3. C_3 can then begin translation of the protein.

mTOR also facilitates the joining of eIF4B to C_3, this greatly enhances the efficiency of the translation process by unwinding the mRNA and speeding it up by a factor of around six times. We denote this faster translation complex as C_4.

As we saw in section 1.2 mutations in NHL cells cause different levels of expression for the signalling pathway components. By building a mathematical model in section 2.2 we hope to see how these mutations could affect the level of capped translation under varing growth factor stimulus. Furthermore, by including the action of the drugs Wortmannin, Rapamycin and ERK-inhibitors we hope to see whether the upregulated capped translation in NHL cells can be reduced to normal levels.

2.2 Mathematical Model

We denote various proteins with variable names shown below

Proteins and variable names					
Growth Factor	G,	PI3K	P,	mTOR	T,
ERK	E,	MEK	M,	Grb2	R,
eIF4E	B^*,	AKT	A,	eIF4B	F,
Drugs and variable names					
Wortmannin	D_1,	Rapamycin	D_2,	PD98059	D_3.

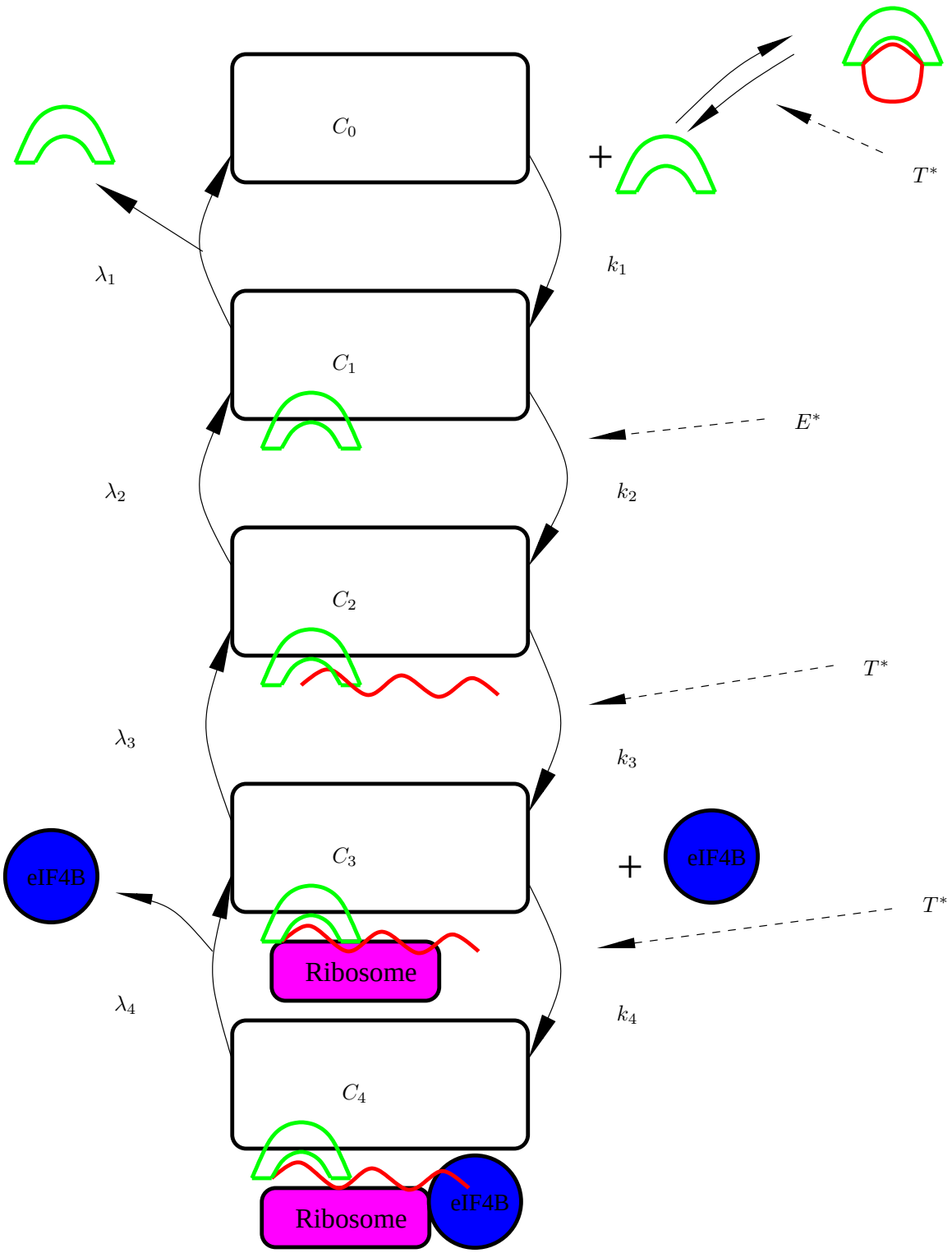

Figure 2: Schematic Diagram of the model. C_0 is the scaffold protein eIF4G/eIF4A. The 'cap' is eIF4E, which can be sequestered by 4E-BP. C_1 is C_0 with the addition of the eIF4E cap. C_2 is C_1 with a target mRNA bound to the cap. C_3 is C_2 with a ribosome in place (the mRNA is now undergoing capped translation). Finally, C_4 is C_3 with eIF4B in place, eIF4A/eIF4B help to 'unwind' the mRNA and speed up translation by a factor of six.

In the formulation of the model that follows a subscript 'T' denotes the total amount of that protein, and a superscript '$*$' denotes an active form of the protein.

We first construct equations to describe the behaviour of the signalling pathways seen in Figure 1, then formulate a model for the formation of the translation complex (Figure 2) under the influence of the pathway targets mTOR and ERK.

P_T gives the total PI3K concentration as active P^* plus inactive P form

$$P_T = P^* + P,$$

similarly for T, A and E:

$$\begin{aligned} T_T &= T^* + T, \\ A_T &= A^* + A, \\ E_T &= E^* + E. \end{aligned}$$

Following Fig. 1 we obtain the following expresions for the ERK pathway. We assume that the proteins reach their equilibrium states quickly and can therefore be represented as constant. There are a number of shared growth factors between the ERK and mTOR pathway, yet also a number for each pathway which are not shared. Here we assume that both are being stimulated by a single shared growth factor 'G'. In the following equations a 'μ' represents a rate of activation/production and a 'v' represents a rate of deactivation/inhibition.

- Grb2 (R) is activated by the growth factor (G) at a rate μ_R

$$R^* = \mu_R G$$

- MEK (M) is activated by the action of active Grb2 at a rate μ_M, but since it is conserved we describe it as

$$M^* = \frac{\mu_M R^* M_T}{1 + \mu_M R^*}$$

- ERK (E) is also conserved, activated by MEK (M^*) at a rate μ_E and inhibited by the drug PD98059 (D_3) at a rate v_{D_3}, giving

$$E^* = \frac{\mu_E M^* E_T}{1 + v_{D_3} D_3 + \mu_E M^*}$$

Following Fig. 1 we now obtain the expresions for the mTOR pathway:

- PI3K (P) is activated by a growth factor, G, at a rate μ_P. Active PI3K is inhibited by the action of the drug Wortmannin (D_1) at a rate v_{D_1}, so we gain the following expression

$$P^* = \frac{\mu_P G}{1 + v_{D_1} D_1}$$

- AKT (A) is conserved and is activated by the action of active PI3K (P^*) at a rate μ_A,

$$A^* = \frac{\mu_A P^* A_T}{1 + \mu_A P^*}$$

- mTOR (T) is conserved and is activated by AKT (A^*) at a rate μ_T. mTOR can be inhibited by the action of Rapamycin (D_2) at a rate v_{D_2},

$$T^* = \frac{\mu_T A^* T_T}{1 + \mu_T A^* + v_{D_2} D_2}$$

Rate	Value	Rate	Value
λ_B	0.5	λ_F	0.5
λ_1	0.5	λ_2	0.25
λ_3	0.15	λ_4	0.15

Table 2: The dissociation rates ('λ's) for the capped complex. All pathway constants ('μ's and 'v's) and association rates ('k's) are defined as unity.

Figure 2 is a schematic of capped translation inititaion, with rates influenced by ERK (E^*) and mTOR (T^*) shown. From Figure 2 we derive the following equations:

- Total cap concentration, [eIF4E], B_T, will be given by the cap concentration plus the concentrations of C_1, C_2, C_3 and C_4

$$B_T = B^* + B + C_1 + C_2 + C_3 + C_4.$$

- Total eIF4B concentration will be the result of active form of eIF4B, F^*, plus the inactive form, F, plus C_4

$$F_T = F^* + F + C_4.$$

- Translation: as described in Section 2.1 we have that translation due to the C_4 complex is six times faster than for the C_3 complex. Thus, we have a measure of translation given by

$$\text{Translation} = C_3 + 6C_4$$

- We assume that C_0 is available in sufficient quantities so as to be assumed constant.

If B^*, F^* denote the concentrations of capped complex and active eIF4B, respectively, then the postulated model is given by the following system of ordinary differential equations derived by the law of mass action from Figure 2:

$$\frac{dB^*}{dt} = k_B T^* B - (\lambda_B + k_1 C_0) B^* + \lambda_1 C_1, \tag{1}$$

$$\frac{dC_1}{dt} = k_1 B^* C_0 - (\lambda_1 + k_2 E^*) C_1 + \lambda_2 C_2, \tag{2}$$

$$\frac{dC_2}{dt} = k_2 E^* C_1 - (\lambda_2 + k_3 T^*) C_2 + \lambda_3 C_3, \tag{3}$$

$$\frac{dC_3}{dt} = -\lambda_3 C_3 + k_3 T^* C_2 - k_4 F^* C_3 + \lambda_4 C_4, \tag{4}$$

$$\frac{dC_4}{dt} = k_4 F^* C_3 - \lambda_4 C_4, \tag{5}$$

$$\frac{dF^*}{dt} = k_F T^* F - (\lambda_F + k_4 C_3) F^* + \lambda_4 C_4. \tag{6}$$

We make a number of assumptions regarding the rates of reaction. The choice of parameters for the given equations are based on the biological data and observations provided by Prof. Anne Willis reseach group. Since we do not have exact parameter values for the given rates and protein concentrations we initially assume that all forward reactions have a unit rate and all reverse reactions occur at a slower rate. The affinity of proteins for the capped complex increases as the complex gains proteins. That is, the complex becomes increasingly stable as we move from C_0 to C_4, and so the rates of dissociation, shown in Table 2, reflect this.

From biological data given we know that there is an up-regulation of proteins AKT, MEK and eIF4B and down regulation of mTOR. From data provided by Prof. Anne Willis reseach group we calculated the ratio increase/ decrease of protein expression between healthy and NHL cells. Thus, in NHL cells we have $A_T = 1.33$, $T_T = 0.69$, $M_T = 1.4$ and $F_T = 2$, where all values are equal to 1 in healthy cells except for F_T which is equal to a half (Table 1).

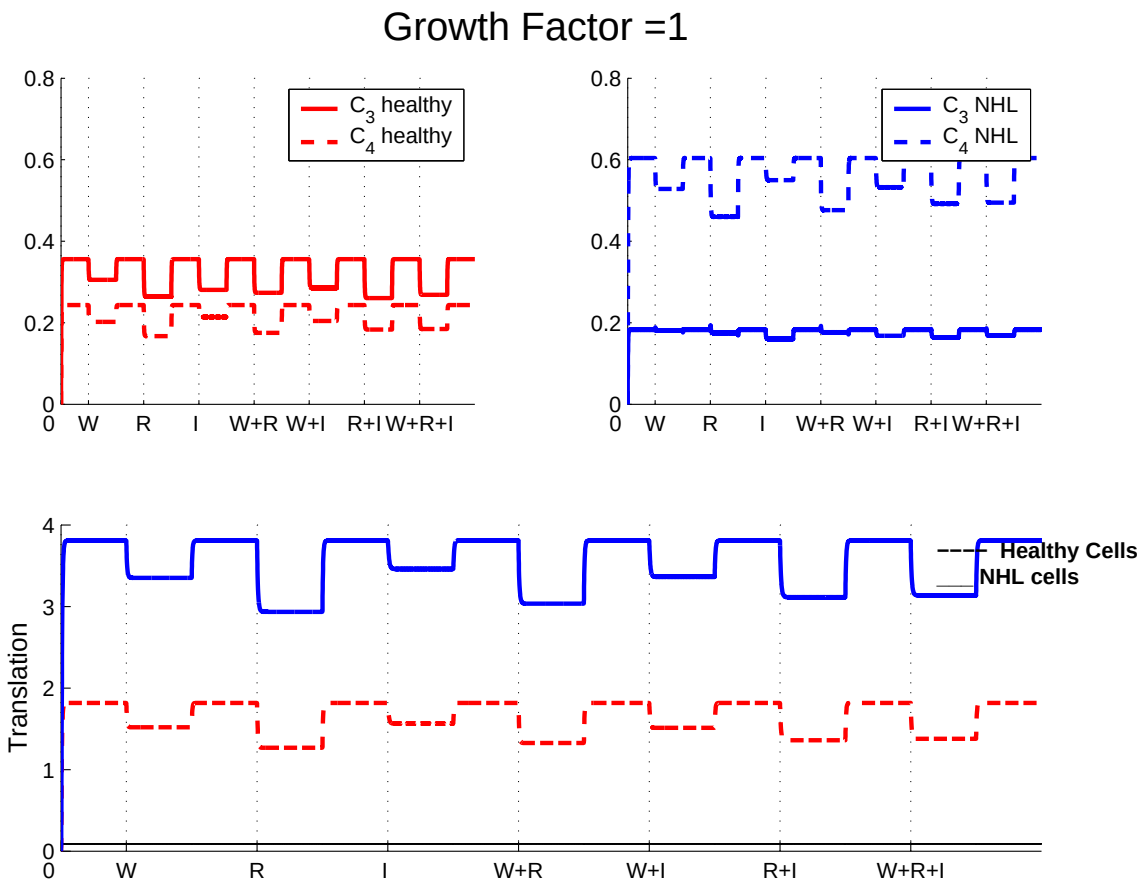

Figure 3: Top two plots consider the activation of C_3 and C_4 complexes in healthy cells (left hand side) and unhealthy cells (right hand side) in unstressed conditions. In healthy cells C_3 is the more abundant complex, whereas in NHL cells we see that the C_3 complexes are nearly half that seen in healthy cells and there is nearly three times the level of C_4 complex present. This is further illustrated in the bottom graph where we see that the rate of translation in NHL cells, is approximately double the rate in healthy cells (dashed line).

3 The results

We solve equations (1)-(6) using the ode23 solver in matlab, a standard ordinary differential equation solver.

We considered the cells under normal, non-stressed conditions where growth factor is unity and approximately 90% of translation is capped (see section 1.1). That is, there are more 'life' proteins than 'death' proteins expressed. When cell becomes stressed and growth factor is reduced then capped translation falls below this level and so the cell expresses more 'death' proteins than 'life' proteins. Using this information we can draw a line representing the point when death protein expression becomes dominant, at 10% of the healthy capped translation level. If the rate of translation decreased to beneath this then cell death pathways would be activated (Figure 3).

We run the same simulation with NHL cells, by simply changing the parameters shown in Table 1. Using results found from healthy conditions we ran simulations looking at stressed cell conditions where we reduced the growth factor ten fold. We keep the same "death threshold" as for high growth factor conditions, by assuming that uncapped translation does not depend on the level of growth factors and occurs at a constant rate (Figure 4).

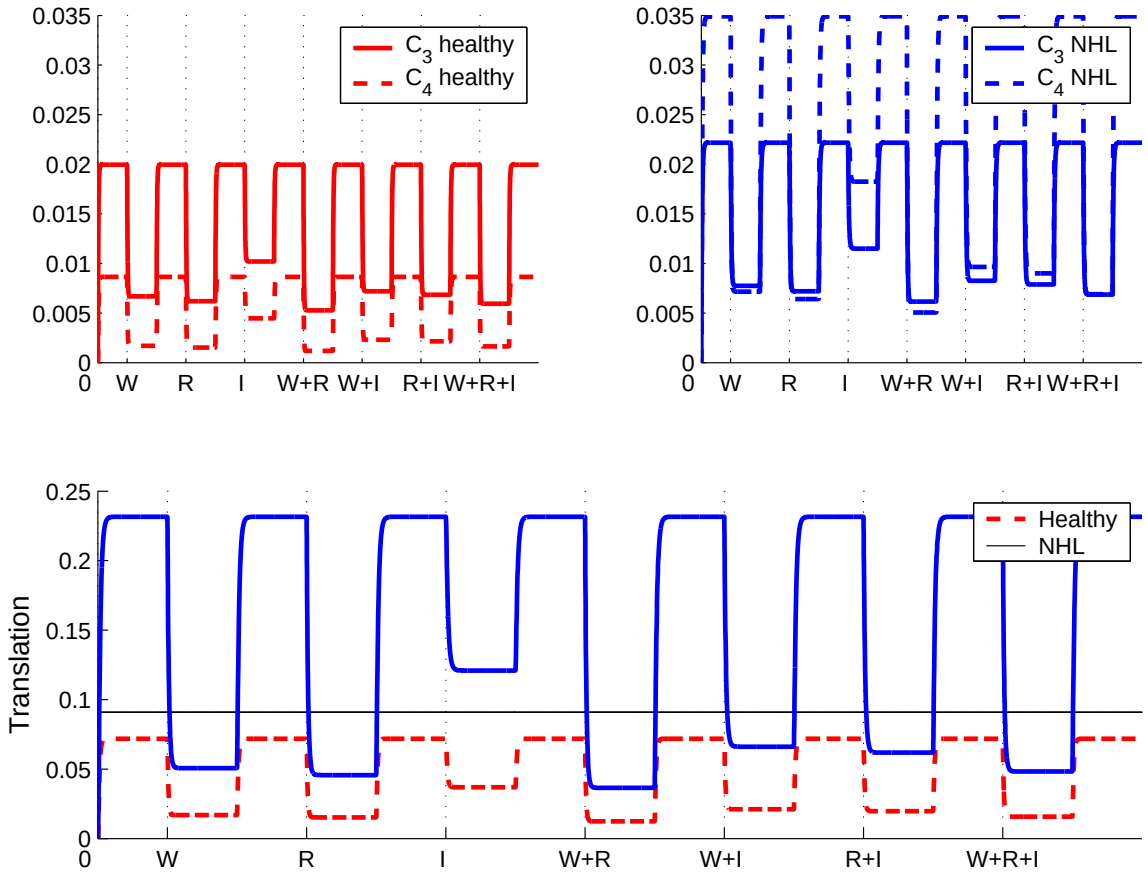

Figure 4: Top two plots consider the activation of C_3 and C_4 complexes in healthy cells (left hand side) and unhealthy cells (right hand side) under stressed conditions. It can be seen that the activation of C_3 complex is only marginally increased for NHL cells where as the activation of C_4 is at least three and a half times that of healthy cells. This is illustrated in the bottom graph where we see that the rate of translation is significantly higher in NHL cells than it is in healthy cells (dashed line).

The plots shown look at the effect of different drugs applied to the cell. We consider three different drugs that act on the pathways at different points. We are considering these drugs on their own and in combination (half the amount of pairs of drugs, and a third the amount of all three) to see which will be most affective in the subsequent treatment regimes.

An ideal treatment regime would have the rate of capped translation in NHL cells falling below the "death threshold" at low growth factor conditions, with the minimum difference in response between healthy and NHL cells.

Interestingly we see that the main effect of NHL mutations is to raise the level of C_4 complex (the more efficient translation machinery) whilst leaving C_3 levels relatively unchanged. This suggests that eIF4B could be another theraputic target.

Each of the plots shown (Figures 3 and 4) first show the affects for no drug administration, where the steady state is obtained. Once the steady state is obtained, which occurs quickly, we add a drug to the system, this will decrease the rate of translation and a different lower steady state is obtained. The drug is then removed from the system so that the rate of translation returns to its original state. We repeat this process applying

different drugs and then combinations of the drugs. The point at which the drug is administered is represented by a dotted vertical line on each of the plots and the drug(s) administered are given by W (Wortmannin, D_1), R (Rapamycin, D_2), and I (PD98059, D_3).

The most important finding is that even at low growth factors, with the addition of some drugs, transcription in NHL cells can be brought down to the transcription levels of healthy cells (Figure 4). Which means that they could behave as healthy cells and this targeting of protein-synthesis is certainly worth investigating experimentally.

As an individual drug, Rapamycin decreases translation the most and hence is the most effective chemosensitizer, we therefore suggest that drugs targeting mTOR will be the most effective. The combination of Rapamycin and Wortmannin is the most effective combination of drugs for reducing the levels of cap dependent translation in the cell. Both of these drugs indirectly affect the activation of mTOR (right hand pathway of Figure 1), so it is clear that the right hand side of the model is the most important when looking at NHL cells.

4 Conclusions

To fully validate our model would require the measurement of many kinetic rates of protein reactions and association, and the estimates of these are the model's main weakness. However its strength is that the only differences between healthy and NHL cells come directly from experimental results.

One of the initial motivations to write down a mathematical model was to stablish if blocking signalling through tranlation inhibition may lead to control of NHL cells, the simulation results show that indeed the addition of drugs to the NHL-cell model reduces the capped translation to a healthy-cell level, with Rapamycin being the most effective individual drug for the given parameters. The model also predicts that capped translation in healthy cells can fall below the level of uncapped translation when growth factors are removed, leading to apoptosis. Furthermore, the model suggests that untreated NHL cells will always have an excess of capped translation and can therefore no longer undergo apoptosis.

We therefore suggest that the pathways controlling capped translation are good drug targets for NHL cells and may allow chemotherapeutic agents to kill NHL cells more easily. The mTOR pathway looks as if it will be the most receptive target, with drugs targeting both mTOR and PI3K being most effective.

References

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