Mathematical model for NF- κ B-driven proliferation of adult neural stem cells

M. J. Piotrowska^{*†,1}, D. Widera^{‡,1}, B. Kaltschmidt[‡], U. an der Heiden^{*}
and C. Kaltschmidt[‡]

**Institute of Mathematics, University of Witten/Herdecke, Stockumer Str. 10, 58448 Witten, Germany, †Institute of Applied Mathematics and Mechanics, Department of Mathematics, Informatics and Mechanics, Warsaw University, Banacha 2, 02–097 Warsaw, Poland, and ‡Institute for Neurobiochemistry, University of Witten/Herdecke, Stockumer Str. 10, 58448 Witten, Germany*

Received 7 April 2006; revision accepted 30 May 2006

Abstract. Neural stem cells (NSCs) are early precursors of neuronal and glial cells. NSCs are capable of generating identical progeny through virtually unlimited numbers of cell divisions (cell proliferation), producing daughter cells committed to differentiation. Nuclear factor kappa B (NF- κ B) is an inducible, ubiquitous transcription factor also expressed in neurones, glia and neural stem cells. Recently, several pieces of evidence have been provided for a central role of NF- κ B in NSC proliferation control. Here, we propose a novel mathematical model for NF- κ B-driven proliferation of NSCs. We have been able to reconstruct the molecular pathway of activation and inactivation of NF- κ B and its influence on cell proliferation by a system of nonlinear ordinary differential equations. Then we use a combination of analytical and numerical techniques to study the model dynamics. The results obtained are illustrated by computer simulations and are, in general, in accordance with biological findings reported by several independent laboratories. The model is able to both explain and predict experimental data. Understanding of proliferation mechanisms in NSCs may provide a novel outlook in both potential use in therapeutic approaches, and basic research as well.

INTRODUCTION

During the development of the central nervous system (CNS), neural stem cells (NSCs) undergo division, cell fate specification and maturation in response to extrinsic cues. In general, stem cells are characterized by the capability to proliferate migrate and differentiate into more specialized cell types, for example, into neurones or glial cells.

Nuclear factor κ B proteins (NF- κ B) are ubiquitous transcription factors crucially involved in regulation of genes relevant in apoptosis and survival, inflammation, cancer development, innate immunity responses (Bonizzi & Karin 2004) (Ghosh & Karin 2002), memory formation

Supported in part by a grant of the Deutsche Forschungsgemeinschaft (DFG) to Christian Kaltschmidt.

Correspondence: Monika Joanna Piotrowska, Universität Witten/Herdecke, Institut für Mathematik, Stockumer Str. 10, 58448 Witten. Tel. +490 2302 669149; Fax: +490 2302 669220; E-mail: monika@mimuw.edu.pl or Monika.Piotrowska@uni-wh.de

¹These authors contributed equally.

Figure 1. Scheme of signal transduction leading to NF- κ B driven proliferation. After binding of an NF- κ B activator (for example, TNF) to its receptor, the signal is transduced to the IKK α/β complex. This then phosphorylates inhibitory I κ B, which is then ubiquitinated and degraded via the proteasome. This degradation triggers the translocation of NF- κ B into the nucleus of the cell, followed by initiation of transcription by binding to regulatory DNA sequences. This leads to transcription of specific target genes and finally to proliferation of NSCs.

and learning (see Kaltschmidt *et al.* 2005 for review). In addition, NF- κ B contributes responsibility for cell growth and proliferation (Guttridge *et al.* 1999; Widera *et al.* 2006a). In the nervous system, the most frequent form of NF- κ B is a heterodimer composed of p50 and p65. The activation of NF- κ B is mainly controlled at the post-transcriptional level by complex formation with the inhibitory protein I κ B in the cytoplasm. Activating stimuli like tumour necrosis factor (TNF) or erythropoietin (EPO) activate a kinase complex composed of two I κ B-specific kinases (IKK α and IKK β) and a modulatory subunit (IKK γ). The IKK- α/β complex phosphorylates the inhibitory I κ B, which is then ubiquitinated and degraded via the proteasome. This degradation triggers the translocation of NF- κ B from the cytoplasm into the nucleus, followed by initiation of transcription and its binding to regulatory DNA-sequences (Baud & Karin 2001), as shown in Fig. 1.

Apart from inducible NF- κ B activity, there are reports on constitutively active NF- κ B in several cell types, such as hippocampal neurones (Kaltschmidt & Kaltschmidt 2000) or numerous cancer cell types (reviewed by Karin 2006). In this study, we focus on inducible NF- κ B activity and the resulting

proliferation of adult neural stem cells. Recently, Mehrhof *et al.* (2005) have convincingly demonstrated genetic evidence for a cell type-restricted requirement of NF- κ B in control of cell proliferation; thus the role of NF- κ B in proliferation has to be analysed for each cell type independently.

Recently we presented evidence for essential involvement of the transcription factor NF- κ B in proliferation control of neural stem cells (reviewed by Widera *et al.* 2006a). We showed that most conditions positively modulated proliferation of NSCs via the NF- κ B pathway. According to experimental and theoretical findings, we propose a novel mathematical model for NF- κ B-dependent proliferation of NSCs. The model suggested here might explain and predict experimental data in basic research concerning cell proliferation control. In addition, it could be of importance for therapeutically used neural stem cell expansion protocols.

MATERIALS AND METHODS

Isolation and culture of adult NSCs

Under a dissection microscope (Zeiss, Jena, Germany), NSCs were isolated from the lateral subventricular zone of adult rats using visually guided micropreparation and were all viewed at $\times 8$ magnification. Isolated tissue containing the stem cell population was digested with 1.33 mg/ml trypsin (Sigma, Deisenhofen, Germany), 0.7 mg/ml hyaluronidase (Sigma, Deisenhofen, Germany), 200 U/ml DNase (Sigma, Deisenhofen, Germany) and 0.2 mg/ml kynurenic acid (Sigma, Deisenhofen, Germany) at 37 °C. Tissue was then passed through a 70- μ m cell strainer (BD Falcon; Heidelberg, Germany) and was transferred to ice-cold, serum-free medium-containing bovine serum albumin (Sigma, Deisenhofen, Germany) to terminate trypsin activity. NSCs were cultured in serum-free media containing basic fibroblast growth factor (bFGF; 20 ng/ml, Chemicon, Hofheim, Germany), epidermal growth factor (EGF; 20 ng/ml; R & D Systems, Wiesbaden, Germany) and B27 supplement (Gibco, Eggenstein, Germany).

Cell number determination

Neural stem cells were plated at 1E6 cells/ml in triplicate for each condition. TNF- α was added to cultures at plating. Spheres were collected at 6, 24, and 48 h after plating, were dissociated and the total cell numbers were counted. Results were expressed as the mean $\pm$ SEM. Statistical significance was determined using two-way ANOVA followed by post-hoc *t*-test with Bonferroni correction. $P \leq 0.05$ was considered statistically significant.

Detection of nuclear NF- κ B in TNF-stimulated neural stem cells

Neurospheres were stimulated with 10 ng/ml TNF for 30 min, harvested via cytospin centrifugation and were fixed with 3.7% formaldehyde for 1 h at room temperature. After 30 min of permeabilization with 0.5% TritonX100/PBS and blocking with 5% goat serum, the cells were stained with activity-specific antip65 antibody (Chemicon, Hofheim, Germany, 1 : 50). Detection was performed by using Cy3-conjugated antimouse antibody (1 : 300, Jackson Immuno Research Laboratories, distributed by Dianova, Hamburg, Germany). Nuclear staining was performed using SYTOX (1 : 10 000, Molecular Probes, Göttingen, Germany). Staining preparations were visualized using confocal laser scanning microscopy (LSM Pascal, Zeiss, Jena, Germany).

Reporter gene assay (transfection and analysis)

Neurospheres were dissociated and transfected using Rat NSC Nucleofector Kit (Amaya, Köln, Germany) according to the manufacturer's instructions; 5E6 cells for each transfection were

used. Transfection efficacy was detected using pmaxGFP vector (Amara) and analysis was performed with fluorescence microscopy (Axiovert 100, Carl Zeiss, Jena, Germany).

For detection of NF- κ B activity in TNF stimulated versus unstimulated neural stem cells, Dual-Luciferase Reporter Assay System (Promega, Mannheim, Germany) and κ B-luc Reporter (BD Clontech, Heidelberg, Germany) were used. All cells were transfected with κ B-luc reporter vector and renilla-luc control vector (Promega, Mannheim, Germany). The cells were lysed and were assayed after 48 h of transfection for promoter dependent luciferase activity versus promoter-independent renilla-luc activity (Lumat LB9507 device, Berthold Technologies, Bad Wildbach, Germany). A representative experiment is shown.

THE MATHEMATICAL MODEL

The changes of active NF- κ B concentration in a cell at time t can be described by the nonlinear ordinary differential equation:

$$\frac{d}{dt}x(t) = \alpha \frac{1 - x(t)}{J_1 + 1 - x(t)} - \beta \frac{\delta(t)x(t)}{J_2 + x(t)} - kx(t), \quad (1)$$

where $x(t)$ and $\delta(t)$ denote the concentrations of active NF- κ B and I κ B at time t , respectively. The J_1, J_2, k, α and β are non-negative constants. Because we assume that the total concentration of NF- κ B is constant and scaled to 1, the difference $(1-x(t))$ represents the fraction of inactive NF- κ B. The first term in Equation 1 represents the activation of NF- κ B via phosphorylation of I κ B by the IKK complex; the second term represents the deactivation of active NF- κ B by NF- κ B-driven autoregulatory expression of I κ B. Both of these reactions are governed by so-called Michaelis–Menten kinetics, and J_1 and J_2 are the Michaelis–Menten constants. The last term in Equation 1 corresponds to the degradation of active NF- κ B.

In a recent study, Hoffmann *et al.* (2002) provided experimental data and numerical results suggesting that there is negative correlation between active NF- κ B and I κ B (compare with Fig. 2 in Hoffmann *et al.* 2002). Thus, we assume that the concentration of I κ B is a decreasing function of active NF- κ B, as represented by a Hill function of $x(t)$

$$\delta(t) = \delta_0 \frac{\theta}{\theta + x(t)}, \quad (2)$$

where δ_0 and θ are positive constants.

In our model we want to determine the change in cell number $y(t)$ of the NSC population. Here we have to take into account that both low and pathologically high NF- κ B concentrations (hyperactivation) lead to cell death in the NSC population (Kaltschmidt *et al.* 2005), and only for intermediate values of $x(t)$ the population increases, compare this with Fig. 2. Therefore, the change in cell number $y(t)$ is described by the differential equation

$$\frac{d}{dt}y(t) = \gamma f(x(t))y(t), \quad (3)$$

with a reproduction function given by

$$f(x(t)) = b^2 - (x(t) - d)^2. \quad (4)$$

Figure 2. Left panel: model for NF- κ B homeostasis (modified after Kaltschmidt *et al.* 2005) linked to proliferation control. The physiological activation state leads to optimal cell growth. Both pathologically high and pathologically low activity, result in apoptotic cell death. Right panel: graph of the corresponding reproduction function $f(x(t)) = b^2 - (x(t) - d)^2$.

The γ , b and d are positive constants. Moreover, b and d have to satisfy $d > b$ and $d + b < 1$ (see Fig. 2). Because of the much larger time scale for cell division than for changes of the concentration variables, we have introduced the rescaling factor γ in the cell growth Equation 3. The factor γ in Equation (3) is unbounded as the cells are cultured *in vitro* and thus are not affected by physiological factors limiting their proliferation, such as cell density or undefined cytokines *in vivo*. In the *in vivo* situation a logistic factor like $\gamma(1-\gamma)$ would be more adequate.

Please note that in the presented model (Equations 1–4) the concentration variables are scaled to dimensionless values.

BASIC PROPERTIES OF THE MODEL, STEADY STATES

Local existence and non-negativity of solutions

By standard arguments from the theory of ordinary differential equations, for each initial condition (x_0, y_0) obeying $x_0 \geq 0$ and $y_0 \geq 0$, there exists a unique local solution $(x(t), y(t))$ satisfying $x(0) = x_0$ and $y(0) = y_0$.

It is obvious that $y = 0$ is the y -nullcline in the (x, y) -phase space for Equations 1–4; therefore $y(t)$ is non-negative wherever $y(0) \geq 0$. Moreover, for $x = 0$ we have $(d/dt)x(t) > 0$, thus $x(t) \geq 0$ whenever $x(0) \geq 0$. Hence, the solutions to Equations 1–4 stay non-negative for non-negative initial conditions (a basic requirement for x and y to represent concentrations).

Boundness of solutions for bounded initial data

Next, we prove that for initial data satisfying $0 \leq x_0 \leq 1$ the solutions to Equation 1 remain less or equal to 1 for as long as they exist (another basic requirement of the model as $x = 1$ means the totality of NF- κ B). From definition (2) it is obvious that for non-negative $x(t)$ we have $0 < \delta(t) < \delta_0$. Using the fact that $x(t)$ is non-negative for non-negative initial data and $\delta(t) > 0$ we can estimate

$$\frac{d}{dt}x(t) = \alpha \frac{1 - x(t)}{J_1 + 1 - x(t)} - \beta \frac{\delta(t)x(t)}{J_2 + x(t)} - kx(t) \leq \alpha \frac{1 - x(t)}{J_1 + 1 - x(t)}. \quad (5)$$

Consider the following equation:

$$\frac{d}{dt}\bar{x}(t) = \alpha \frac{1 - \bar{x}(t)}{J_1 + 1 - \bar{x}(t)}. \quad (6)$$

Assume $\bar{x}(0) = x(0) \leq 1$. If the solution $\bar{x}$ to Equation 6 would become greater than 1, then there would exist $t^* \geq 0$ such that $\bar{x}(t^*) = 1$ and $(d/dt)\bar{x}(t^*) > 0$. Contradictorily, it follows from (6) that $(d/dt)\bar{x}(t^*) = 0$. Therefore the solution $\bar{x}$ to Equation 6 must stay less or equal to 1. Because of the estimation provided by Equation 5, the same must hold also for the solution x to Equation 1.

Global existence

It follows from $0 \leq x(t) \leq 1$

$$\frac{d}{dt}x(t) \leq \alpha \frac{1 - x(t)}{J_1 + 1 - x(t)} \leq \frac{\alpha}{J_1} (1 - x(t)) \leq \frac{\alpha}{J_1},$$

It is obvious that the solution to equation $(d/dt)x(t) = \alpha/J_1$ has the form $x(t) = x(0) + (\alpha/J_1)t$ and it exists for all $t \geq 0$, therefore the solution to Equation 1 also exists for all $t \geq 0$ provided $0 \leq x(0) \leq 1$.

The function $f(x)$ has the maximal value $f(x_{\max}) = b^2$ value for $x_{\max} = d$. We estimate Equation 3 as follows:

$$\frac{d}{dt}y(t) = \gamma f(x(t))y(t) \leq \gamma b^2 y(t).$$

Using the same technique as in case of Equation 1, we are able to prove the global existence of solutions to Equation 3.

Steady states and their stability

Throughout the following, we fix all values of the parameters with the exception of the constant α , see Table 1. The solutions of Equations 1–4 strongly depend on the constants d and b .

Table 1. Parameter values for Equations 1–4

Parameter	Value
α	0–5
β	4.5
γ	0.013
δ_0	1.5
θ	0.1
b	0.19
d	0.73
k	0.045
J_1	3
J_2	12

Figure 3. Steady state $x(t) = x_3$ (for all t) as a function of the parameter α (NF- κ B activator, for example, TNF) for Equation 1. The basal set of parameter values is given in Table 1.

For the following investigation we chose the parameters b and d according to the conditions $0 < d - b < d + b < 1$. In particular, for the calculations we chose $b = 0.19$ and $d = 0.73$.

Whenever $\alpha \neq 0.5244$ and $\alpha \neq 3.408$ (meaning that the solutions to equation $(d/dt)x(t) = 0$ are unequal to $d - b$ or $d + b$), then for the parameter values presented in Table 1, the system equations 1–4 has four steady states $(x_1, y) = (x_1, 0)$, $(x_2, 0)$, $(x_3, 0)$ and $(x_4, 0)$. By numerical computation it is easy to see that x_1 is always negative and less than -10 , $x_2 \in (-2, 0)$, $x_3 \in [0, 1]$ and $x_4 > 3$ (compare with Fig. 3). Because the total concentration of NF- κ B is normalized to 1, the only biologically relevant steady state is $(x_3, y) = (x_3, 0)$. Note that if $\alpha = 0.5244$ or $\alpha = 3.408$, then we obtain the straight lines of steady states $(x_3, y) = (d - b, y) = (0.54, y)$ and $(x_3, y) = (d + b, y) = (0.92, y)$, respectively.

The location of the x -nullcline (x_3, y) strongly depends on the constant α . It is clear that if $\alpha = 0$, then $x_3 = 0$. If we increase the value of α then the value of the steady state level x_3 increases up to 0.9452 for $\alpha = 5$ (compare with Fig. 3).

By stability analysis we see that for $\alpha \in (0, 0.5244)$ or $\alpha \in (3.408, 5)$ (that is, $x_3 \in (0, d - b)$ and $x_3 \in (d + b, 1)$, respectively) the steady state $(x_3, 0)$ to Equations 1–4 is a stable node, however, for $\alpha \in (0.5244, 3.408)$ $(x_3, 0)$ is a saddle point. In the first case, the population of NSCs will generally die out, whereas in the second case the population will grow to infinity. For the singular values $\alpha = 0.5244$ and $\alpha = 3.408$ the steady state $(x_3, 0)$ is a non-hyperbolic point.

TIME-DEPENDENT SOLUTIONS

In this section, we first present some numerical solutions of the model defined by Equations 1–4. We focus on the influence of the parameter α (extrinsic stimulus) on the behaviour of solutions

Figure 4. Graph of $\beta\delta_0\theta x/(\theta + x)(J_2 + x)$ (green) and kx (red) for $x \in [0,1]$, constant values as in Table 1.

$(x(t),y(t))$. For all calculations, we fixed the parameters according to Table 1, only changing the magnitude of α . As an initial condition for Equations 1–4, we take $x(0) = 0.1$ (relative value), namely relatively low basal amount of active NF- κ B in the beginning, and $y(0) = 1$, the initial number (in millions) of cells cultured. All calculations were performed for the time period provided in minutes from 0 to 4320.

Because in a cell, the active NF- κ B is mainly inactivated by I κ B and only a relatively small amount of active NF- κ B is proteasomally degraded, we chose the constants k and β such that for all $x \in [0,1]$ the inequality

$$\frac{\beta\delta_0\theta x}{(\theta + x)(J_2 + x)} > kx,$$

holds; compare this with Fig. 4.

In this study, we would like to show the effect of activating stimuli (for example, TNF) on NF- κ B as well as the influence of this stimulus on the population of NSCs. In Fig. 5, the changes of the behaviour of solutions x to Equation 1 with respect to different values of the parameter α are presented. We see that for very small α (red) there is weak activation of NF- κ B. In such a case there would not be sufficient active NF- κ B and the cells would not be able to proliferate (compare the red line in Fig. 5 to the red line in Fig. 6). If we increase the value of α (green), then the concentration of active NF- κ B would be sufficient and cells would start to divide. Further increase of α will lead to increased proliferation. However, if α were to be too high, then NF- κ B would be hyperactivated and consequently cells would undergo apoptosis (compare the blue lines in Figs 5 and 6).

Figure 5. Graph of x -solution to Equations 1–4 for different values α : 0.25 (red), 1.1 (green) and 3.6 (blue), with initial condition $x(0) = 0.1$. All other parameter values as in Table 1. The variable x represents NF- κ B activity, α stands for relative concentration of the NF- κ B activator, e.g. TNF.

Figure 7 shows the concentration of I κ B defined by Equation 2 for different values of the parameter α . We see that with increasing α , the concentration of I κ B decreases. Moreover, for slower activation of NF- κ B, the degradation of I κ B is also slower (compare lines red, green and blue in Figs 5 and 7). Figure 8 presents the y -solution to Equations 1–4 in time for parameter values α in the interval $[0.5]$. This simulation shows a characteristic hump. For small values of the stimulus α we obtain a decreasing number of NSCs, which finally become extinguished. If the stimulus is strong enough, it activates a sufficient amount of NF- κ B and cells start to proliferate. For still larger α , the number of cells increases more rapidly. If α is very large, the stimulus activates too much NF- κ B, causing the slower proliferation of NSCs. However, for extremely large α , the y -solution to Equations 1–4 decays to 0 in time.

Figure 9 shows in three dimensions how the x -solutions to Equation 1 depend on different values of the parameter α (the concentration of the stimulus, for example, TNF) and time t . In accordance with our model, TNF has recently been identified as mitogen in the subventricular zone of rats. Stereotactical injection of TNF- α in the lateral ventricle has resulted in a highly significant increase of proliferating cells 24 h post-injection (Wu *et al.* 2000). Fig. 10 shows a comparison of experimental *in vitro* data and model 1–4 predictions. Figure 10a indicates a total number of NSCs after exposure to 10 ng/ml TNF. As predicted in the model, adult neural stem cells respond to TNF with significantly increased proliferation compared to untreated controls. In addition, TNF treatment leads to activation of the NF- κ B pathway as shown by nuclear translocation of NF- κ B visualized using an activity dependent antibody against the p65 subunit. Moreover, NF- κ B activation by TNF in neural stem cells became evident as increased κ B bioluminescence. A comparison of experimental data and the model simulation is provided in

Figure 6. Graph of y -solution to Equations 1–4 for different values α : 0.25 (red), 1.1 (green) and 3.6 (blue), with initial condition $x(0) = 0.1$, $y(0) = 1$. All other parameter values are as in Table 1. The variable y represents the relative cell number, α stands for relative concentration of the NF- κ B activator, for example, TNF.

Fig. 10d. As before, the parameter values used in these simulations are shown in Table 1. The orange line is the number of cells (in millions) predicted by our model for $\alpha = 0.9$. This line corresponds to the control (that is, cell culture without the supplementary dose of TNF), when the cell number was determined in the experiment (orange rhomb).

Here, we are able to note that the behaviour of the model, described in previous discussions, is not restricted to the chosen parameter values of Table 1. Simulations with other biologically reasonable values showed very similar behaviour solutions. Moreover, with general considerations, one can show that these qualities hold for a broad range of parameters.

DISCUSSION

In this study we have developed a novel model for NF- κ B-driven proliferation of adult neural stem cells. We propose that in NSCs, there is strict correlation between NF- κ B activity and cell proliferation. In this context it is noteworthy that several investigations have demonstrated a neuroprotective role of NF- κ B in pathological situations, requiring endogenous regeneration via increased proliferation of neural stem cells (Kaltschmidt *et al.* 1999b; Fridmacher *et al.* 2003; Marchetti *et al.* 2004). According to these findings, it has recently been proposed that the NF- κ B transcriptional pathway plays a crucial role in proliferation control of NSCs (review by Widera *et al.* 2006a).

Figure 7. Graph of $\delta(t)$ for different values α : 0.25 (red), 1.1 (green) and 3.6 (blue), x given by solution to Equation 1 with initial condition $x(0) = 0.1$. All other parameter values as in Table 1. The function $\delta(t)$ represents the relative concentration of I κ B. α stands for relative concentration of the NF- κ B activator, for example, TNF.

Cell number

Figure 8. Graph of y -solutions to Equations 1–4 for different values of parameter $\alpha \in [0, 5]$. Simulation was performed for the initial data $x(0) = 0.1, y(0) = 1$. All other parameter values are as in Table 1. The variable y represents the relative cell number, α stands for relative concentration of the NF- κ B activator, for example, TNF.

Figure 9. Graph of x -solutions to Equation 1 for different values of parameter $\alpha \in [0,5]$. The simulation was performed for the initial data $x(0) = 0.1, y(0) = 1$. All other parameter values are as in Table 1. The variable x represents the NF- κ B activity, α stands for relative concentration of the NF- κ B activator, such as TNF.

With regard to control of NF- κ B activity, Hoffman *et al.* (2002) proposed a mathematical model for the NF- κ B activity and its regulation by the inhibitory I κ B protein. The experimental data and numerical results presented indicated that there is negative correlation between active NF- κ B and I κ B (see Fig. 2 in Hoffmann *et al.* 2002). Thus, in our model, we have assumed that the concentration of I κ B is a decreasing function of active NF- κ B given by equation 2, compare also Figs 5 and 7.

After binding of the stimulating agent (e.g. TNF, here α) to the receptor, the signal is transduced via intermediate kinases to the IKK α/β complex. This leads to phosphorylation of I κ B and finally to its degradation. Thus, in our model we assume that activation of inactive NF- κ B ($1 - x(t)$) is governed by the Michaelis–Menten kinetics. This is described by the first term in Equation 1.

Demonstrably, NF- κ B can also be activated in a receptor independent manner by conditions such as hypoxia (Jung *et al.* 2003), hyperthermia or ultraviolet exposure (Perkins 2004). The

Figure 10. Comparison of experimental data and model Equation 1–4. (a) Shows total number of NSCs after exposure to 10 ng/ml TNF. As predicted in the model, adult neural stem cells respond to TNF with significantly increased proliferation compared to untreated controls. (b) Indicates that TNF treatment leads to activation of the NF- κ B pathway as shown by nuclear translocation of NF- κ B, visualized using an activity dependent antibody against the p65 subunit (taken with permission from Widera *et al.* 2006b). (c) Reveals that NF- κ B activation by TNF in neural stem cells became evident as increased bioluminescence of a κ B dependent reporter plasmid. Relative bioluminescence is shown as the ratio of luminescence of NF- κ B luciferase vector/luminescence of CMV-renilla luciferase vector. (d) Is the comparison of experimental data and y -solution to Equations 1–4 for $\alpha = 0.9$ (orange) and $\alpha = 1.09$ (green). The orange line is the number of cells (in millions) predicted by our model for $\alpha = 0.9$. This line corresponds to the control (that is, cell culture without the supplementary dose of TNF), cell number measured experimentally (orange rhomb). Green dots represent cell numbers obtained after treatment of NSC with TNF. The green line is the result of simulation of Equations 1–4 with parameter $\alpha = 1.09$.

degradation of I κ B leads to nuclear translocation of NF- κ B, followed by binding to the regulatory DNA sequences and finally to transcription of the target genes.

It is notable that our model differs from the models proposed by Hoffmann *et al.* (2002) and Cheong *et al.* (2006) or Lipniacki *et al.* (2004). First, we do not consider explicitly various molecules or molecular complexes such as bimolecular IKK-I κ B, I κ B-NF- κ B and trimolecular IKK-I κ B-NF- κ B for each I κ B isoform (α , β and ϵ). In our model we focus on interaction between the IKK complex and inactive NF- κ B, I κ B and active NF- κ B. We also consider proteosomal degradation of active NF- κ B. This simplification allows us to propose a mathematical model that consists of two non-linear ordinary differential equations with 8 constants instead of more than 20 equations with more than 30 independent model parameters, as Hoffmann *et al.* (2002) or Cheong *et al.* (2006). Moreover, in the first equation of the presented model, the activation and deactivation of NF- κ B are formulated as Michaelis–Menten rate laws, and in consequence we obtain a different type of non-linearity than in equations formulated by Hoffmann *et al.* (2002), Cheong *et al.* (2006) or Lipniacki *et al.* (2004). Additionally, our proposed model describes the influence of active NF- κ B on adult NSCs proliferation.

Interestingly, *in vivo* NF- κ B activity must be regulated very accurately. Pathologically high activity leads to apoptotic cell death; too low activity results in the same phenomenon (Kaltschmidt *et al.* 2005). In that text with this in mind, Kaltschmidt *et al.* (2005) remind us of Paracelsus' 'All things are poison and nothing (is) without poison; only the dose makes that a thing is poison or not'. Thus, cell proliferation and cell death are regulated in a manner similar to a classical bell-shaped curve (compare Fig. 2 and our definition of reproduction function, Equation 4). We would like to point out that several recent studies provide experimental (Singh *et al.* 2004) and theoretical evidence (Ganguly & Puri 2006) for the so called cancer stem cell hypothesis. Demonstrably, proliferation of cancer cells is mainly NF- κ B dependent (Hinz *et al.* 1999; Kaltschmidt *et al.* 1999a). Thus, one can hypothesize that our model might have impact for both stem cell research and with minor modification for cancer research.

Taken together, the present model may provide a potent tool for prediction of results of proliferation assays and NSC expansion approaches for clinical use. Our results have generated a simulation of a physiological process regulated by NF- κ B activation, namely that of cell proliferation driven by NF- κ B.

ACKNOWLEDGEMENTS

This paper was prepared within the framework of the EU project Modelling, Mathematical Methods and Computer Simulation of Tumour Growth and Therapy, MRTN – CT – 2004 – 503661.

REFERENCES

- Baud V, Karin M (2001) Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol.* **11**, 372–377.
- Bonizzi G, Karin M (2004) The two NF- κ B activation pathways and their role in innate and adaptive immunity. *Trends Immunol.* **25**, 280–288.
- Cheong R, Bergmann A, Werner SL, Regal J, Hoffmann A, Levchenko A (2006) Transient I- κ B kinase activity mediates temporal NF- κ B dynamics in response to a wide range of tumor necrosis factor- α doses. *J. Biol. Chem.* **281**, 2945–2950.
- Fridmacher V, Kaltschmidt B, Goudeau B, Ndiaye D, Rossi FM, Pfeiffer J, Kaltschmidt C, Israel A, Memet S (2003)

- Forebrain-specific neuronal inhibition of nuclear factor-kappaB activity leads to loss of neuroprotection. *J. Neurosci.* **23**, 9403–9408.
- Ganguly R, Puri IK (2006) Mathematical model for the cancer stem cell hypothesis. *Cell Prolif.* **39**, 3–14.
- Ghosh S, Karin M (2002) Missing pieces in the NF-kappaB puzzle. *Cell* **109** (Suppl.), S81–S96.
- Guttridge DC, Albanese C, Reuther JY, Pestell RG, Baldwin AS Jr (1999) NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol. Cell. Biol.* **19**, 5785–5799.
- Hinz M, Krappmann D, Eichten A, Heder A, Scheidereit C, Strauss M (1999) NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. *Mol. Cell. Biol.* **19**, 2690–2698.
- Hoffmann A, Levchenko A, Scott ML, Baltimore D (2002) The IkappaB-NF-kappaB signaling module: temporal control and selective gene activation. *Science* **298**, 1241–1245.
- Jung Y, Isaacs JS, Lee S, Trepel J, Liu ZG, Neckers L (2003) Hypoxia-inducible factor induction by tumour necrosis factor in normoxic cells requires receptor-interacting protein-dependent nuclear factor kappa B activation. *Biochem. J.* **370**, 1011–1017.
- Kaltschmidt B, Kaltschmidt C (2000) Constitutive NF-kappa B activity is modulated via neuron–astroglia interaction. *Exp. Brain Res.* **130**, 100–104.
- Kaltschmidt B, Kaltschmidt C, Hohner SP, Droge W, Schmitz ML (1999a) Repression of NF-kappaB impairs HeLa cell proliferation by functional interference with cell cycle checkpoint regulators. *Oncogene* **18**, 3213–3225.
- Kaltschmidt B, Uhrek M, Wellmann H, Volk B, Kaltschmidt C (1999b) Inhibition of NF-kB potentiates amyloid- β -mediated neuronal apoptosis. *Proc. Natl. Acad. Sci. USA* **96**, 9409–9414.
- Kaltschmidt B, Widera D, Kaltschmidt C (2005) Signaling via NF-kappaB in the nervous system. *Biochim. Biophys. Acta* **1745**, 287–299.
- Karin M (2006) NF-kappaB and cancer: mechanisms and targets. *Mol. Carcinog.* **45**, 355 [Epub ahead of print].
- Lipniacki T, Paszek P, Brasier AR, Luxon B, Kimmel M (2004) Mathematical model of NF-kappaB regulatory module. *J. Theor. Biol.* **228**, 195–215.
- Marchetti L, Klein M, Schlett K, Pfizenmaier K, Eisel UL (2004) Tumor necrosis factor (TNF) -mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. *J. Biol. Chem.* **279**, 32869–32881.
- Mehrfhof FB, Schmidt-Ullrich R, Dietz R, Scheidereit C (2005) Regulation of vascular smooth muscle cell proliferation: role of NF-kappaB revisited. *Circ. Res.* **96**, 958–964.
- Perkins ND (2004) Regulation of NF-kappaB by atypical activators and tumour suppressors. *Biochem. Soc. Trans.* **32**, 936–939.
- Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB (2004) Identification of human brain tumour-initiating cells. *Nature* **432**, 396–401.
- Widera D, Mikenberg I, Kaus A, Kaltschmidt C, Kaltschmidt B (2006a) Nuclear factor-kappaB controls the reaggregation of 3D neurosphere cultures. *In Vitro* **11**, 76–84.
- Widera D, Mikenberg I, Kaltschmidt B, Kaltschmidt C (2006b) Potential role of NF-kappaB in adult neural stem cells: the underrated steersman? *Int. J. Dev. Neurosci.* **24**, 91–102.
- Wu JP, Kuo JS, Liu YL, Tzeng SF (2000) Tumor necrosis factor-alpha modulates the proliferation of neural progenitors in the subventricular/ventricular zone of adult rat brain. *Neurosci. Lett.* **292**, 203–206.