Aberration in NFκB–IκB binding may cause two states of NFκB activity

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NFκB activation is involved in cell survival and aberration of NFκB signalling is found in cancer. IκB is an inhibitor that sequesters NFκB in the cytoplasm. By mathematical modelling, we predict that under an aberrant condition in which IκB does not bind to NFκB, there exist two states of NFκB activity: a constitutive activity state for any positive value of the stimulus and a zero activity state for no stimulus. Further, under the assumption that NIK can be activated by TAB1–pTAK1, we predict that TAB1 has a role in the basal activity of NFκB. Thus, we predict the importance of TAB1 and IκB in NFκB activation. Our prediction may have implication for cell survival and cancer.

Keywords: Basal activity, cancer, cell survival, constitutive activation, protein complex.

PREVIOUSLY, others and the present author[1] have described the network that causes p38 activation in response to IL1. A similar network[2], which shares many proteins with the IL1/p38 network, can also cause the nuclear accumulation of NFκB in response to IL1. Starting with the IL1 receptor till the activation of TAK1, the two networks share most of the proteins and diverge at TAK1. There are two mechanisms of TAK1 activation by IL1. The first mechanism involves TAB2 and the ubiquitin ligase TRAF6 (ref. 3), while the second involves TAB1. The first mechanism is the ubiquitination-dependent activation of TAK1, while the second mechanism may be a phosphorylation-dependent activation of TAK1 (refs 4, 5). It is not clear whether both mechanisms can cause NFκB accumulation in the nucleus. TAK1 makes a complex, called TRAF6-regulated IKK activator (TRIKA2), with TAB1 and TAB2 (ref. 6). Although the TRIKA2 complex activated IKK in the presence of ubiquitin-conjugating enzymes Ubc13 and Uev1A and the ubiquitin ligase TRAF6, the role of TAB1 in this complex is not clear[7]. TAK1 phosphorylates NIK, which activates IKK[2]. Since role of TAB1 in TAK1 and NIK activation is not clear, we have considered both cases: (i) NIK can associate with pTAK1 activated by TAB2–TRAF6, but not with pTAK1 activated by TAB1 and (ii) NIK can associate with both pTAK1 phosphorylated by TAB2–TRAF6 and by TAB1. Active IKK phosphorylates IκB, leading to its proteasomal degradation, which causes accumulation of NFκB in the nucleus.

IκB is a cellular inhibitor of NFκB[8]. IκB sequesters NFκB in the cytoplasm and prevents NFκB-induced gene transcription. Aberration in IκB production, degradation and phosphorylation has been implicated in many diseases[9]. For this reason, we considered NFκB accumulation in the nucleus also in the absence of NFκB–IκB binding reaction.

Taking into account the reactions of IL1/p38 and IL1/NFκB networks, we constructed a mathematical model of NFκB activation by IL1. The model shows that under normal conditions, NFκB is activated transiently over several hours and finally achieves a steady state. Under the aberrant condition, in which IκB does not bind to NFκB, we found that the cells have two states. The first state is the zero NFκB activity state which occurs under zero concentration of IL-1. On the other hand, in the second state, a constitutive activation of NFκB is achieved for any positive stimulation. Further, we found that under the assumption in which NIK can associate and be activated by TAB1-activated TAK1, the basal activity of NIK and NFκB is affected, which is similar to our earlier prediction that TAB1 affects the basal activity of p38 (ref. 1).

The method used in the mathematical modelling of IL1-induced NFκB signalling network has been described in detail in the earlier work[1]. Briefly, in the IL1-induced p38 signalling network[1], we have added a module to model activation of NFκB (Figure 1). Reactions of this module have been modelled using the law of mass action. All reactions of IL1-induced NFκB signalling network

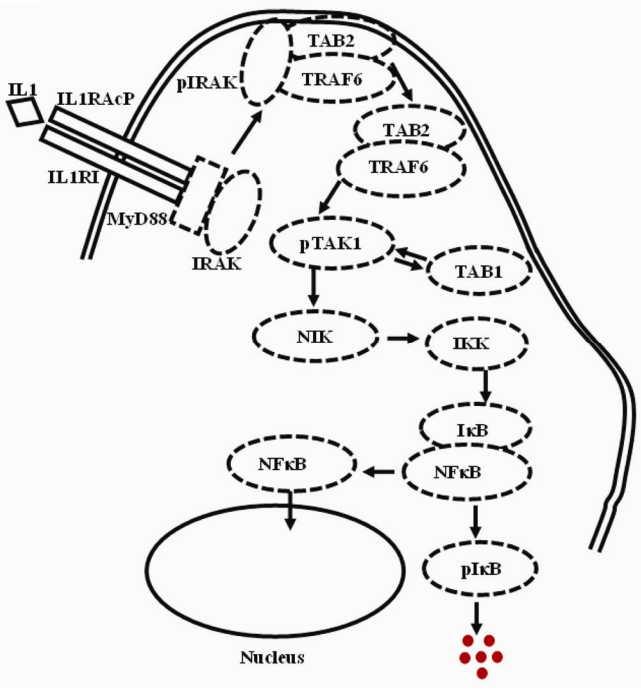

Figure 1. IL1/NFκB activation network. IL1-induced degradation of IκB and nuclear translocation of NFκB are shown.

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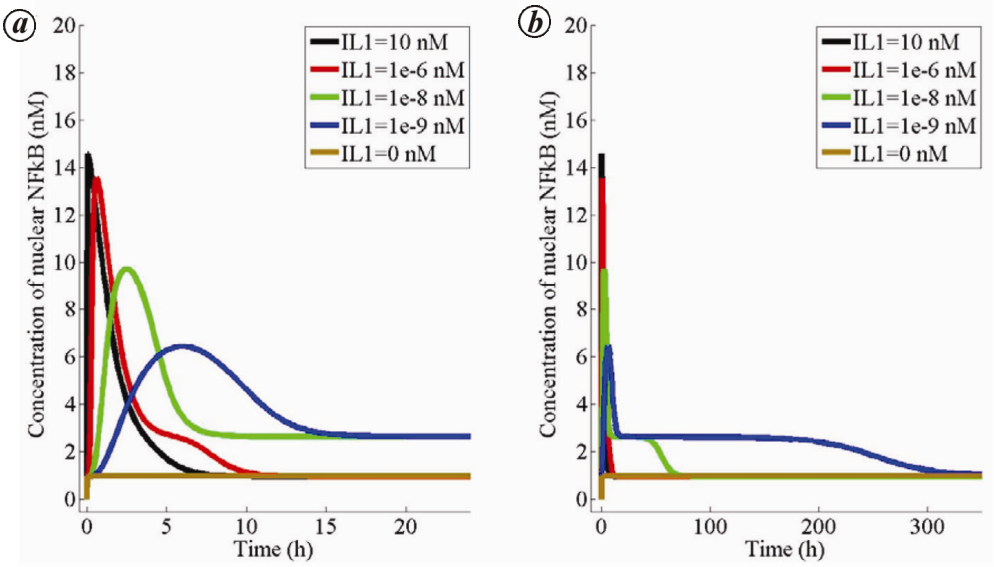

Figure 2. IL1 causes transient accumulation of NFκB in the nucleus. It has been assumed that TAK1, activated by TAB1, cannot activate NIK.

and the rate constants are given in the 'Supporting Information'. A cell has been modelled as a batch reactor using the batch reactor design equation

$$\frac{dC_i}{dt} = \sum_j r_{ij},$$

where C_i is the concentration of a protein/protein complex in the IL1/NFκB signalling network and r_{ij} is the rate of formation of the species i in the jth reaction. The resulting set of ordinary differential equations has been solved using the ode solver ODE15s of MATLAB R2010b (Math Works, MA, USA) with initial conditions, which are given in the Supporting Information.

First, we investigated nuclear accumulation of NFκB over a 24 h period and found that the NFκB response consists of a transient phase of 5–10 h duration followed by a steady state (Figure 2 *a* and *b*). The steady state is independent of IL1 concentration (Figure 2 *b*). As IL1 concentration decreases, the peak of NFκB transient phase becomes lower and gets delayed. This response is similar to that of p38 in the presence of IL-1, although p38 activation happens within an hour[1] and is thus more rapid.

Since IκB is required for the sequestration of NFκB in the cytoplasm, we explored the dynamics of NFκB nuclear accumulation when NFκB–IκB binding reaction is disrupted. We found that every concentration of IL1 causes constitutive accumulation of NFκB in the nucleus (Figure 3 *a*). As the concentration of IL1 decreases, the response gets delayed although the steady state remains the same (Figure 3 *b* and *c*). In the absence of IL1, NFκB nuclear accumulation is zero. Thus even a small perturbation of cells, in the form of IL1 stimulus, causes constitutive nuclear accumulation of NFκB. This binary response can be seen as cells having two steady states: one in the presence of the stimulus and the other in its absence. This all-or-none response resembles ON/OFF states of an enzyme. In the absence of IkB binding to NFκB, the latter is primed to translocate to the nucleus. Any small perturbation in the form of IL1 stimulus can cause this to happen.

TAK1 can be activated by TAB2-TRAF6 in an IL1-dependent manner or by TAB1 in an IL1-independent manner[4]. It is not clear whether TAK1 activated by both mechanisms can cause nuclear accumulation of NFκB. Thus, we investigated NFκB nuclear response in the presence and absence of NIK activation by TAB1. We first considered TAK1 activation by IL1 in the presence and absence of NIK activation by TAB1 and found that like p38 (ref. 1) and NFκB nuclear responses, TAK1 activation consists of a transient phase and a basal activity and is independent of whether or not NIK is activated by TAB1 (Figure 4 *a–d*). TAK1 activation is also independent of whether or not NFκB–IκB binding reaction has been disrupted (Figure 4 *a* and *c*), which is expected since TAK1 activation is upstream of NFκB–IκB binding reaction.

Next, we studied NIK activation by IL1 in the presence or absence of such activation by TAB1 and in the presence or absence of NFκB–IκB binding reaction. Similar to pTAK1, NIK activation also consists of a transient phase and a steady state (Figure 5 *a–d*). Regarding the effect of TAB1, we found that it affects only the basal activity of NIK (Figure 5 *b* and *d*). Further, we did not find any effect of NFκB–IκB binding reaction on NIK activation (Figure 5 *a–d*), which is expected since NIK activation is upstream of NFκB–IκB binding reaction.

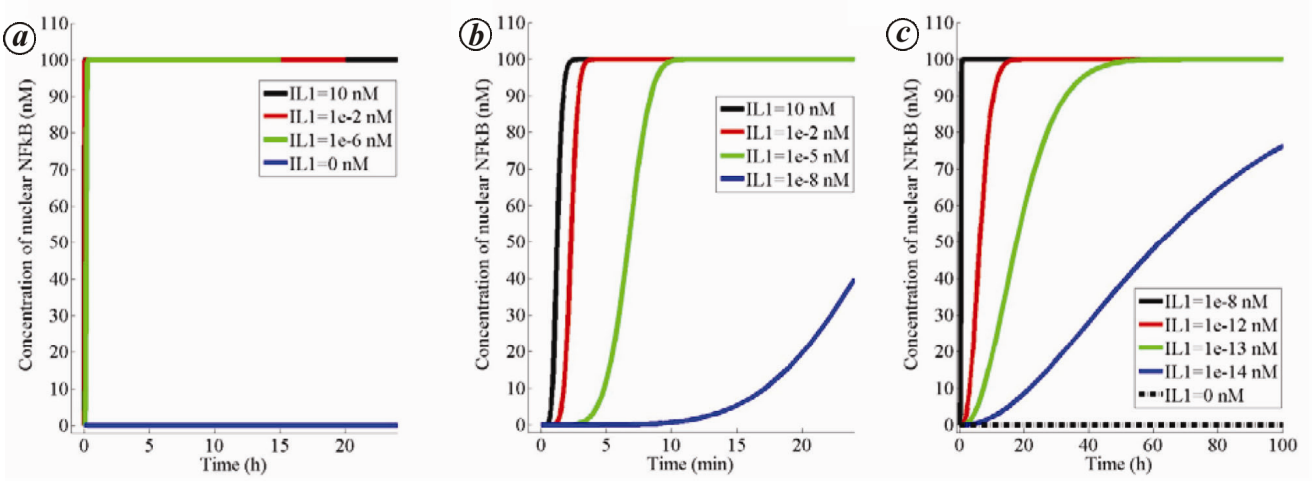

Figure 3. Absence of IκB binding with NFκB causes constitutive accumulation of NFκB in the nucleus. It has been assumed that TAK1, activated by TAB1, cannot activate NIK. Further, it has been assumed that due to aberration IκB cannot bind with NFκB.

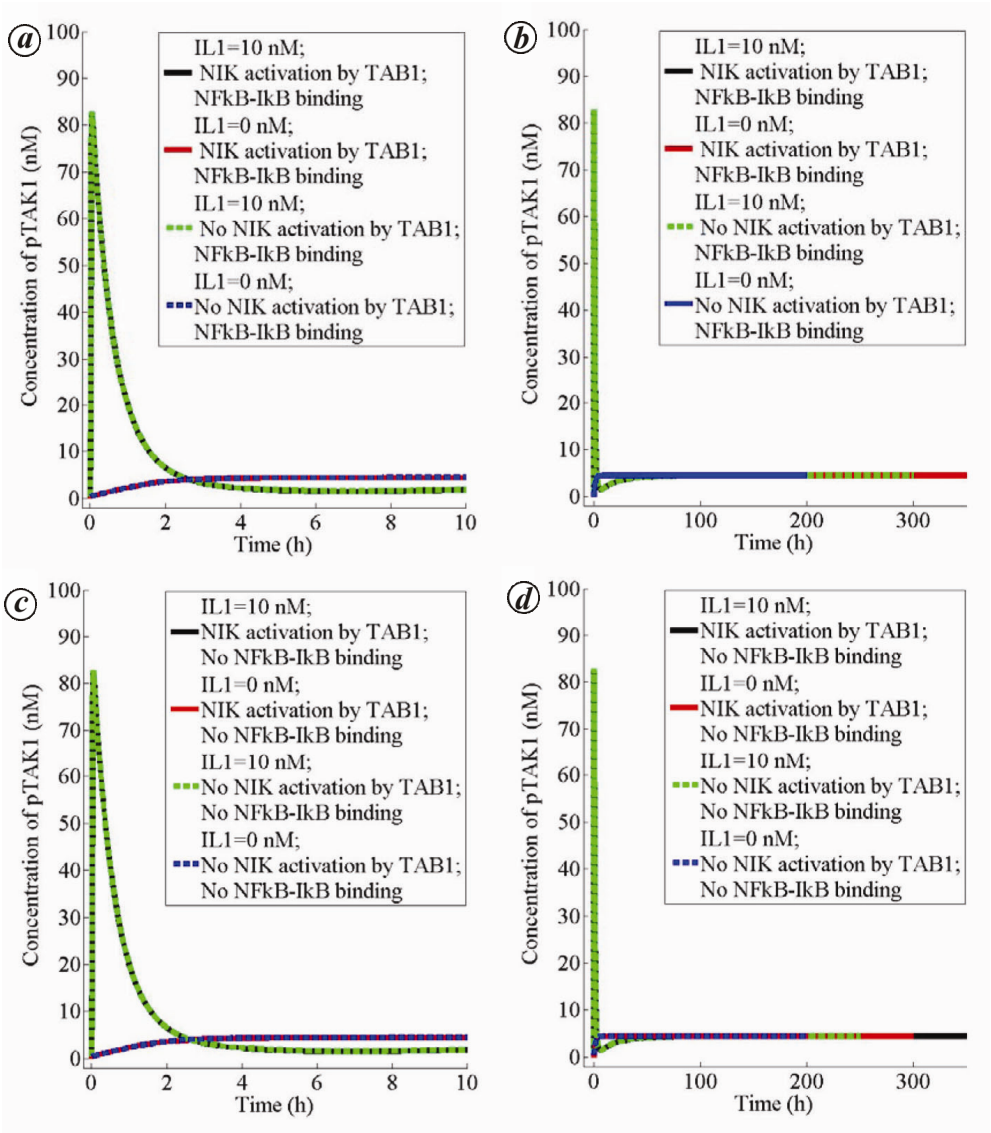

Figure 4. TAK1 activation is not affected by TAB1–pTAK1–NIK interaction and NFκB–IκB binding reaction.

We studied the role of TAB1 in nuclear accumulation of NFκB, both in the presence and absence of the NFκB–IκB binding reaction and found that NIK activation by TAB1 affects only the basal accumulation of NFκB in the nucleus in the presence of the binding reaction (Figure 6 *c* and *d*). On the other hand, in the absence of NFκB–IκB interaction and presence of NIK activation by TAB1–pTAK1, all of the NFκB is found in the nucleus (Figure 6 *a*), suggesting that under this aberrant condition NFκB can accumulate in the nucleus even in the absence of the stimulus. Further, in the absence of both the NIK activation by TAB1–pTAK1 and the NFκB–IκB interaction, NFκB is not activated in the absence of the stimulus (Figure 6 *b*), suggesting that the stimulus-independent activation of NFκB occurs only in the aberrant condition in which NFκB–IκB interaction is abolished and NIK can be activated by TAB1-pTAK1. In the absence of the NFκB–IκB binding reaction, NIK (thus TAB1) is not connected to the NFkB nuclear import (Figure 1). Thus, under the aberrant condition, NIK and TAB1 have no role in the nuclear accumulation of NFkB, and NFkB accumulates maximally in the nucleus.

We investigated the effect of TAB1 on the dynamics of nuclear NFκB, which shows that increasing TAB1 level has no effect on the dynamics of NFκB (Figure 7 *a* and *b*), if TAB1 does not activate NIK. On the other hand, if TAB1 can activate NIK through pTAK1, TAB1 increases the basal level of nuclear NFκB (Figure 7 *c* and *d*), suggesting a role of TAB1 in the basal activity of NFκB.

Previously, we modelled and predicted the time course of nuclear activation of p38 in response to IL-1, and showed that p38 activation peaks at around 2 min and reaches a basal level within an hour. Nuclear accumulation of NFκB also includes a transient phase followed by a steady state. However, NFκB translocation to the nucleus is slower and reaches a basal level within 5 h. Like p38, the peak of NFκB accumulation in the nucleus is delayed as the IL-1 concentration is decreased and the dynamics is robust to changes in IL-1 concentration. However, dynamics of nuclear NFκB is much more robust to changes in the IL-1 concentration, since reduction of IL-1 over seven orders of magnitude has only minor effect on the dynamics of NFκB. For a concentration of 10 nM, the peak of NFκB activity is attained at around 3 min while for a concentration of 10^{-6} nM, the peak is attained at around 35 min. Thus, in the physiological concentration range 1–10 pg/ml or 57e-6–57e-5 nM, the peak is attained within 35 min (ref. 10). A concentration of 10^{-8} nM may cause around ten-fold activation (relative to the basal activity) of NFκB. However, at this concentration of IL-1, the peak is attained at around 2.5 h and attainment of basal activity may take up to 70 h of treatment with IL-1. Thus relative to p38 activation, NFκB activation is more sustained. In support of our prediction, Rasmussen *et al.*[11] studied the time course of NFκB activation due to IL-1 in HepG2 cells. They showed that there is a basal level of nuclear NFκB. Further, they showed that NFκB peaks within 15 min of addition of IL-1 and activation continues for more than 24 h in the presence of IL-1. Similarly, Moynagh *et al.*[12] studied the dynamics of NFκB activation by IL-1 in C6 glioma cells, and found that it is activated within 20 min and activation continues up to 24 h. However, we do not see a clear variation of NFκB activity at different time points in the study of Moynagh *et al.*[12]. It is possible that in their case, the NFκB detection assay might have been saturated.

Since in some cancer cell lines NFκB has been found to be constitutively active[13,14], we explored the dynamics of NFκB in the absence of binding of its inhibitor IκB. The binding of NFκB–IκB may be abolished due to the aberrant transcription or mutations in the IκB gene. Our model predicts that under this condition the cells have two states: (1) a zero nuclear NFκB activity under no stimulation condition, and (2) a constitutive nuclear NFκB activity under any non-zero stimulation condition. Thus, stimulation may be considered as a perturbation to the cells, and a cell maintains any of the two states.

Previously, we have shown that TAB1 regulates the basal activity in p38 activation. In the p38 activation we assumed that TAK1 activated both by TAB2–TRAF6 and TAB1, can activate p38. However, due to possible involvement of ubiquitin, it is not clear whether TAK1 activated by TAB1 can also activate NIK. Therefore, we chose two conditions: (i) NIK is activated by pTAK1 activated by TAB2–TRAF6, but not by TAB1, and (ii) NIK is activated by pTAK1 phosphorylated both by TAB2–TRAF6 and TAB1. By analysing these two conditions, we predict that TAB1 may regulate the basal activity of both NIK and NFκB. In support of our prediction, Neil *et al.*[15] reported a truncated TAB1 in 4T1 cells, which decreased both the basal and TGF-β-induced activity of NFκB. Similarly, Lu *et al.*[16] studied NFκB activation in the absence of a stimulus and found that X-linked inhibitor of apoptosis (XIAP) induces NFκB activity through interaction with TAB1, implicating TAB1 in the basal activity of NFκB.

We investigated the condition under which NFκB will be constitutively active. Two conditions resulted. In the first condition, in which NFκB–IκB binding reaction has been abolished and pTAK1 activated by TAB1 can activate NIK, NFκB is constitutively active. In the second condition, in which NFκB–IκB binding reaction has been abolished but pTAK1 activated by TAB1 cannot activate NIK, NFκB is active for all positive stimulation while NFκB activity is zero for no stimulation condition. Experimentally, both conditions will result in constitutive activation of NFκB, since a positive stimulation in terms of a perturbation will be always present in the microenvironment of a cell. Thus, disruption of NFκB–IκB binding reaction may be the aberrant condition that may occur in some cancers.

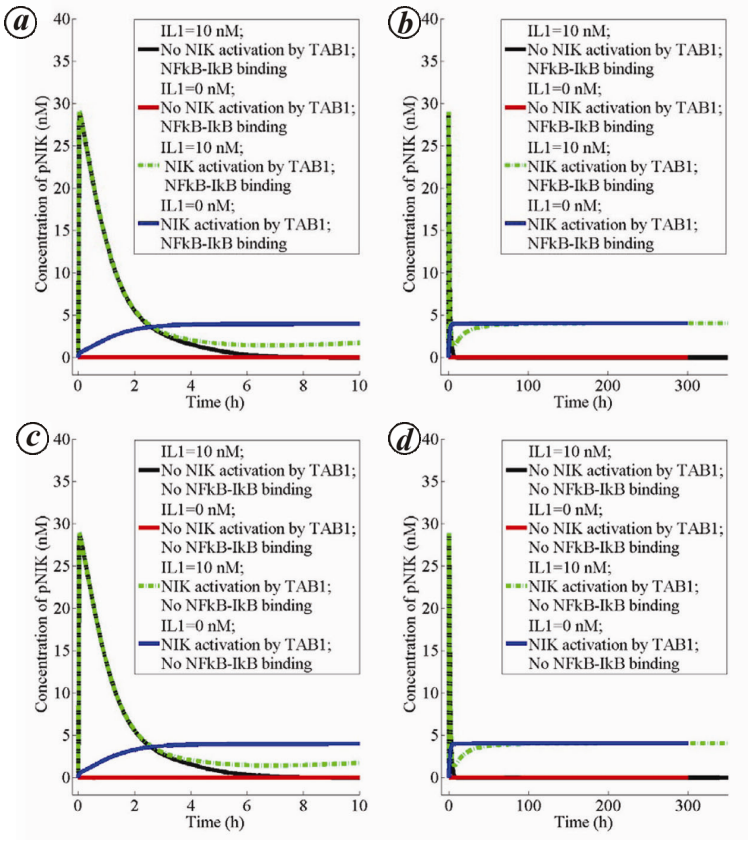

Figure 5. Basal activity of NIK is affected by TAB1.

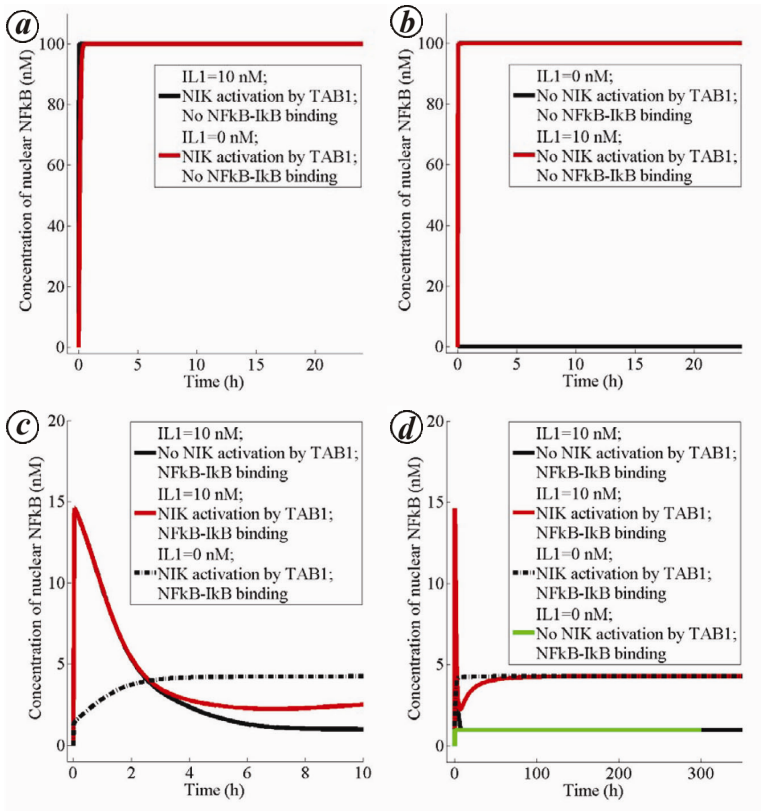

Figure 6. NIK activation by TAB1–pTAK1 causes constitutive activation of NFκB in absence of NFκB–IκB interaction.

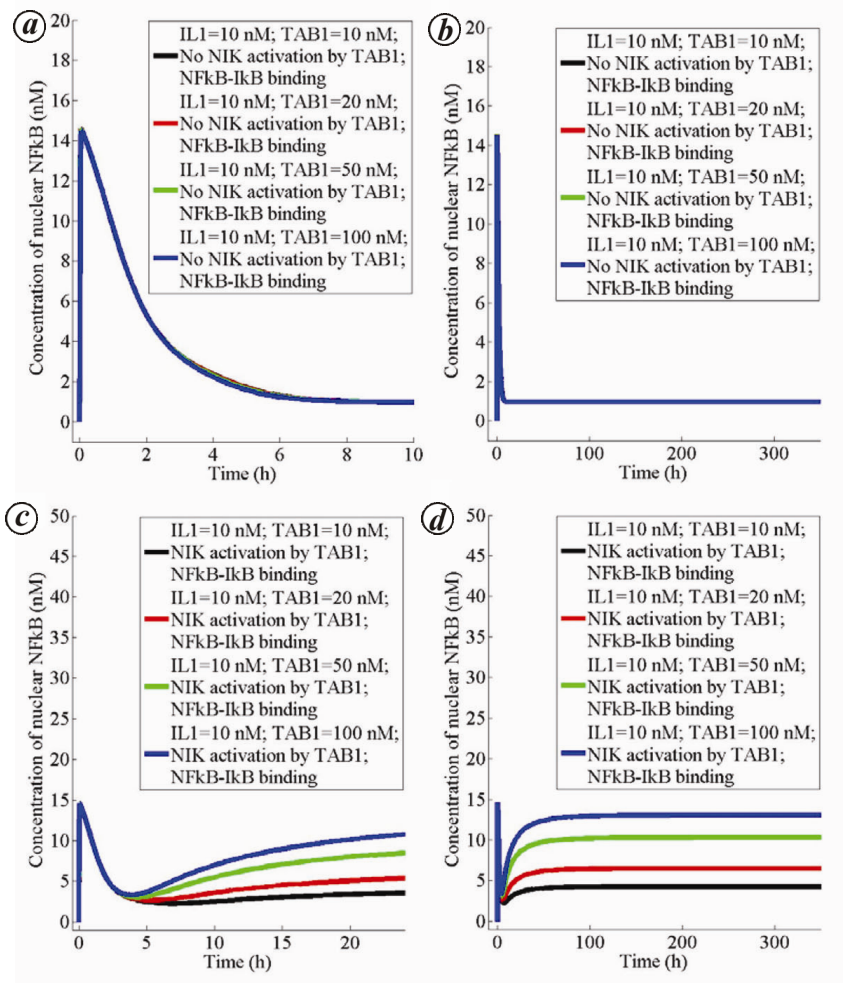

Figure 7. TAB1 affects the basal activity of NFκB.

NFκB has roles in many diseases including inflammatory and metabolic diseases[17,18], autoimmune diseases[19], cancer[20] and HIV latency[21]. In HIV latency, IkBα has been found to inhibit HIV replication by inhibiting HIV–Rev transactivation inside the infected cells[21]. In cancer, several mutations in proteins in the NFκB signalling network have been identified[20]. Besides, NFκB activation has been found in the underlying inflammation leading to cancers[20]. Similarly, among the autoimmune diseases, NFκB has been found to affect type-I diabetes[22]. Thus, our study has implications for several diseases, including cancer.

In summary, TAB1 may regulate the basal activity of NFκB. Further, in the absence of NFκB–IκB binding as well as NIK activation by TAB1-pTAK1, NFκB may have two states – an all or none kind of activation. All of NFκB will be in the nucleus for any stimulation and none will be present in the nucleus for zero stimulation. On the other hand, in the absence of NFκB–IκB binding and in the presence of NIK activation by TAB1–pTAK1, NFκB is constitutively active.

Conflict of interest. The author declares that he has no conflict of interest.

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Experimental analysis of the ratio of similar materials by similarity model test on raw coal

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Similarity model test is an effective approach to study the mechanism of hydraulic fracture propagation in coalbed methane reservoirs as well as theoretical analysis and numerical simulation. The efficiency of the similarity model test result is closely related to the selection and ratio of similar materials. Similar material ratio test was conducted to simulate the mechanical parameters of raw coal using orthogonal method and an appropriate similarity model for hydraulic fracturing experiment was developed in this study. Results show that it is suitable to select cement, gypsum as binder and apply pulverized coal as aggregate through the analysis of experimental data. The mechanical parameters of similar materials, including uniaxial compressive strength, elastic modulus, Poisson ratio and firmness coefficient are tested using laboratory tests. The impact of diverse ratios of similar materials on the mechanical parameters is analysed. A proper ratio is selected to make the mechanical parameters of raw coal close to the ones of similar material, in order to meet the demand of the similarity model test based on raw coal. The results can provide theoretical basis and technical support for the selection of similar materials to carry out hydraulic fracturing experiments.

Keywords: Experimental investigation, hydraulic fracturing, raw coal, similar materials, mechanical parameters.

COALBED methane (CBM) reserves are abundant in China, ranking third in the world, but have the characteristics of low permeability, saturation and porosity, which make it difficult to realize commercial development of CBM[1]. Hydraulic fracturing technology can effectively improve coal reservoir permeability, and prevent coal and gas outburst[2–5]. Hydraulic fractures are the main channel for CBM; the efficiency of hydraulic fracturing depends

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