The Construction of Mathematical Model for the Mechanism of Protein Synthesis Involving mTORC1 from the AMPK Pathway

Ari Kusumastuti, Mohammad Jamhuri, Dewi Firdaus and Nurul Anggraeni Hidayati

***Abstract*—This study discusses the construction of mathematical models for the mechanism of protein synthesis involving the main regulator mTORC1 gene which is described in the singular mTOR pathway. The novelty of this research is taking the AMPK pathway. The genes included, i.e., AMPK, TSC2, Rheb, mTORC1 and S6K. The method used for this research is divided into two stages, namely pathway analysis for the mechanism of protein synthesis and the second is the formulation of mathematical models. Pathway analysis is performed as a reference in describing interactions in the form of kinetic reaction schemes. After the interaction scheme is created, it is then formulated into a mathematical model with the independent variable being time. Mathematical models for the mechanism of protein synthesis involving mTORC1 of the AMPK pathway in the form of ordinary time-dependent differential equations involving independent variables $[TSC2]$, $[pAMPK]$, $[pTSC2]$, $[Rheb^{GTP}]$, $[Rheb^{GDP}]$, $[mTORC1]$, $[Raptor]$, $[aRaptor]$, $[Deptor]$, $[PRAS40]$, $[mTOR]$, $[amTORC1]$, $[mLST8]$, $[S6K1]$ and $[pS6K1]$. The mathematical models for mechanism of protein synthesis involving mTORC1 of the AMPK pathway is used to understanding the complex dynamics of biochemical signaling cascades on the mTOR pathway.**

***Index Terms*—Mathematical modelling, Protein Synthesis, Kinetic Reaction.**

I. Introduction

MODELING Mathematics is the essential tool to bridge the various kinds of scientific discipline toward the certain focuses of interest to be explore together. For example, understanding the disorder metabolic which leads to degenerative diseases, such as type2 diabetes mellitus (T2DM). This research focuses on how to formulate mathematics model in ordinary differential equation (ODE) depend on time for protein synthesis mechanism within mTORC1 pathway that is related to the type 2 diabetes mellitus (T2DM). The pathway to guide this research can be seen in the https://www.genome.jp/pathway/hsa04150.

AMPK, TSC1/2, Rheb, mTORC1, S6K1 as the regulator genes and used as main variables of this research. Here is the theoretical background of the variables.

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A. AMPK

AMP-activated protein kinase (AMPK) has a important role in metabolism control. AMPK often being target for metabolic disorders curative [1]. AMPK is a heterotrimeric serine/threonine kinase that made from α, β and γ subunits, which have each role on AMPK activity. From these subunits, multiple isoforms are formed, i.e. $\alpha_1, \alpha_2, \beta_1, \beta_2, \gamma_1, \gamma_2$, and γ_3. There are 12 AMPK complexes can be formed from these isoforms with different combinations and different flavors[2]. The eukaryotes cellular energy level cultivation done by these 12 AMPK complexes.

The regulation of AMPK complexes performed by AMP:ATP or ADP:ATP ratios changing. The number of bioceuticals and diabetes medications such as metformin and thiazolinonediones also have an effect to AMPK regulation. Phosphorylation AMPK by three kinases and dephosphorylation by phosphatases are being controller of AMPK biochemical activity.

Many of pharmaceutical companies still trying to discover firsthand AMPK activator in order to create cardiovascular and metabolic diseases medication. Over the last few years, a huge advances in the AMPK structural biology was made and being a beginning of detailed molecular providing. At last, topology of fascinating enzymes is discovered little by little. The binding of small molecules cause conformational changes that leads to the activation and protection from the dephosphorylation. In a short review of AMPK structure and function, the molecular interactions of firsthand synthetic AMPK activators make an effect to AMPK isoform activation [3].

B. TSC2

The Tuberous sclerosis1/2 (TSC1/2) complex trans-locate into lysosomes to inactivate mTORC1. This localization is a response from two stresses, i.e. amino-acid starvation and growth factor removal. These two stresses are required to maintain TSC2 cytoplasmic simultaneously. If one of these stresses is missing, localization TSC2 to lysosomes will be happened right away. The inhibitor of mTORC1 also make an effect to TSC2 lysosomal accumulation. This accumulation is a general response to stimuli inactivate mTORC1. Any single stress can cause TSC2 lysosomal localization.

TSC is well known as important inhibitor of mTORC1 activity, which is composed from TSC1, TSC2 and TBC1D7 proteins. As part of the complex, TSC2 has GTPase-activating

protein (GAP) activity towards Rheb, and catalyze the conversion from the active GTP-bound state into inactive GDP-bound conformation. All the stimuli that control mTORC1 activity converge on TSC2 to regulate the function [4].

C. Rheb

Rheb belongs to a unique family within the Ras superfamily of G-proteins. Rheb was identified in rat brain for the first time, then known that Rheb is conserved from yeast to human. The lower eukaryotes only have one Rheb, while mammalian cells have two Rheb.

The function of Rheb is activate mTOR to growth. In particular, this ability is needed on direct the interaction of Rheb with mTORC1 complex. Rheb is one of monomeric proteins with the size approximately $20-30$ kDa. Beside binds guanine nucleotides, Rheb have function as a molecular switch by shuttling between GTP-bound and GDP-bound forms.

The Ras superfamily consists of Ras, Rho, Rab, Arf, and Ran with Ras serving as the founding subfamily. These proteins control some pathways, such as adhesion, traffic, motility, transport, transformation, cellular growth, and signal transduction. GTP hydrolysis is upgraded by GAPs (GTPase activator proteins), beyond intrinsic GTPase activity [1], [5]. GAPs keep Ras superfamily proteins in the inactive GDP-bound state. On the other hand, GEFs (Guanine nucleotide exchange factors) interceded the switching of GDP to GTP [6], [7]. GEFs keeps the proteins in the active GTP-bound state. GDIs (GDP dissaciation inhibitors) is an additional set of proteins that act as an extra layer of regulation, which inhibit nucleotide switching by bind to the GDP-bound form [8].

D. mTORC1

The remains residue from Rheb phosporylates Raptor limit mTORC1 activity. Activating mTOR means mTOR can modulate blocked Raptor activity by rapamycin. mTOR activity can be blocked by PRAS40 so the binding of mTORC1 to Raptor is disturbed. PRAS40 is released from Raptor if the phosphorylation by protein kinase B (Akt) is happened. From this, PRAS40 will be separated to mTORC1 activating activity by the cytoplasmic docking protein $14-3-3$. The binding Deptor and FAT domain of mTOR also can inhibit mTORC1 activity. Beside, mLST8 support mTORC1 activity. The p70 ribosomal S6 kinase (p70S6K) and the eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1) will bind to Raptor so mTORC1 activity will not be inhibited. Nevertheless, the binding Raptor to p70S6k and 4EBP1 can be blocked by PRAS40. For additional information, mLST8 supports mTORC1 activity and also known as insulin signaling controller. The controlling done through the transcription factor FoxO3, be necessary for Akt and protein kinase C-α (PKCα) phosphorylation, and is required for the association between Rictor and mTOR [9].

E. Serine/threonine

Serine/threonine is a kind of protein kinase that acts downstream of mTOR signaling. This protein works as responses to growth factors and nutrients to promote cell proliferation, cell growth, and cell cycle development. The role of serine/threonine is on regulating protein synthesis (through EIF4B, RPS6, and EEF2K phosphorylation) and surviving cell by repress the pro-apoptotic function of BAD. Serine/threonine works under nutrient depletion and the inactive form associates with the EIF3 translation initiation complex. After mitogenic stimulation, mTORC1 phosphorylation by the mammalian target leads to dissociation and activation from the EIF3 complex. Several pre-initiation complex substrates also being phosphorylated and activated by the active EIF3. Those complex such as EIF2B complex and the cap-binding complex component EIF4B. Serine/threonine also have a role on controlling translation initiation. The role done by phosphorylating a negative regulator of EIF4 and PDCD4, targeting these for ubiquitination and subsequent proteolysis. Besides, serine/threonine phosphorylates POLDIP3/SKAR in order to promoting initiation the pioneer round of protein synthesis. Serine phosphorylates EEF2K for translation elongation activating. Which is can inhibit IGF1 and activate EEF2. Serine also has a role on feedback regulation of mTORC2 by mTORC1 with phosphorylating RICTOR [10].

II. IDENTIFY TYPES OF INTERACTIONS AMONG GENES

A. Interaction between AMPK and TSC2

The fact that AMPK activates TSC2 has been proven in [11] and supported by the Software String analysis data shown in Fig. 1, which has the highest score of 0.861. Chen further stated that AMPK's interaction with TSC2 is inhibiting the mTORC1 signal as an important gene agent in protein synthesis.

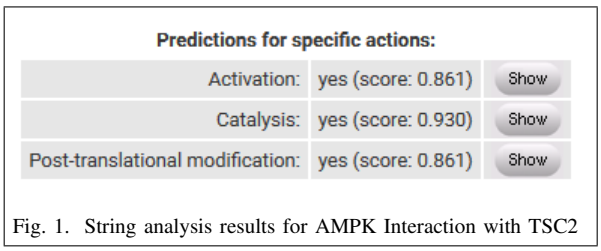

Fig. 1. String analysis results for AMPK Interaction with TSC2

B. Interaction between TSC2 and Rheb

Based on the analysis of TSC2 and Rheb String Software has an inhibition relationship (inhibitory) with a score of 0.953 where TSC2 as an inhibitor or inhibitor of Rheb, especially in the conversion of Rheb GTP to Rheb GDP. This relationship has been proven by [7], [8] and by the results of software String analysis. TSC have function as Rheb's GAP and hydrolysis accelerator of Rheb-GTP on the lysosome surface to an inactive Rheb-GDP[7]. TSC1/TSC2 heterodimer which consists of TSC1 and TSC2 gene products functions as a GTPase activating protein for Rheb conversion of GTP bound forms to GDP bound forms [8].

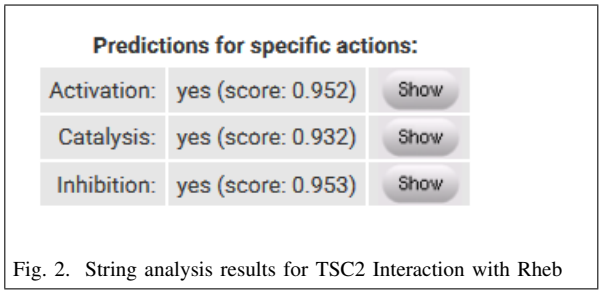

Fig. 2. String analysis results for TSC2 Interaction with Rheb

C. Interaction between Rheb and mTORC1

Rheb and mTOR have binding interactions or bindings between the two, as evidenced by [8], [12], and Software String analysis with binding scores of 0.959. mTORC1 kinase activity increases when mTOR binds to active Rheb (Rheb GTP) [12]. In addition, no activation was observed when Rheb was tied to GDP [8].

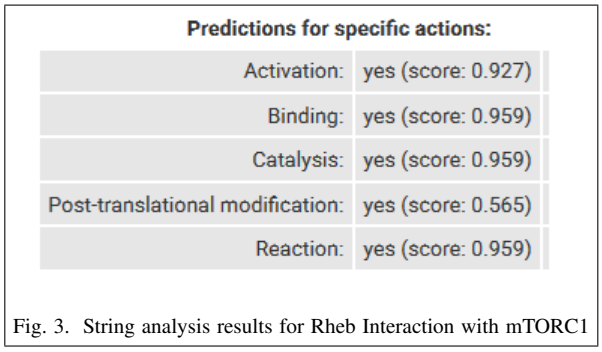

Fig. 3. String analysis results for Rheb Interaction with mTORC1

D. Interaction between PRAS40 and mTOR

The fact that PRAS40 inhibits mTOR is proven by [9] and the results of Software String analysis with a score of 0.845. The binding of mTOR to the Raptor can be inhibited by PRAS40 [9].

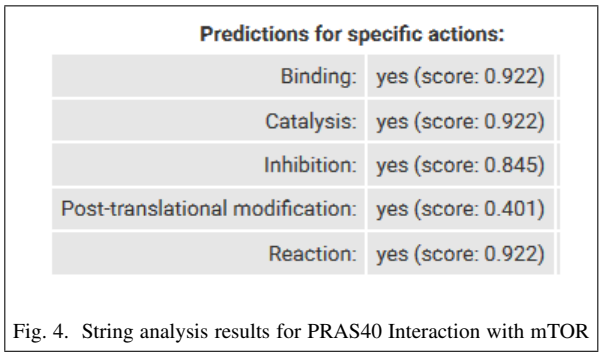

Fig. 4. String analysis results for PRAS40 Interaction with mTOR

E. Interaction between PRAS40 and Raptor

The fact that PRAS40 inhibits Raptor is proven by [9], [13] and Software String analysis with a score of 0.809. PRAS40 binds raptor in vitro and in vivo [13]. PRAS40 inhibits mTOR activity and inhibits binding of mTOR with Raptor [9]. PRAS40 can increase the activity of mTORC1 kinase when it is phosphorylated by protein kinase B (Akt) [9]. So it can be concluded that the interaction from PRAS40 to Raptor is binding then inhibits Raptor binding with mTOR.

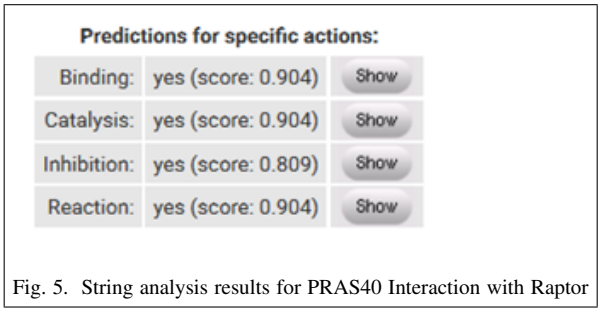

Fig. 5. String analysis results for PRAS40 Interaction with Raptor

F. Interaction between Deptor and mTOR

The fact that the Deptor inhibits mTOR is proven by [9], [14]. The Software String analysis in Fig. 6 with an inhibitory score of 0.804. Deptor binds to mTOR FAT domain, so make mTORC1 delayed [9]. On the extracellular metabolic and inflammatory processes, all depend to mTOR signaling as central regulator, whereas DEP containing proteins that interact with mTOR (DEPTOR) is a natural inhibitor of mTOR [14].

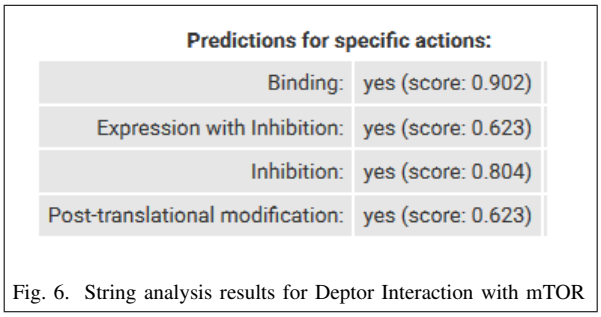

Fig. 6. String analysis results for Deptor Interaction with mTOR

G. Interaction between Rheb and Raptor

The fact that Rheb activates the Raptor is proven by [9] and the Software String analysis in Fig. 7 with a score of 0.917. [9] mentions that Rheb phosphorylates Raptor serine863 residues and other residues that include serine859, serine855, serine877, serine696, and threonine706. Rheb phosphorylation of the Raptor makes the Raptor active. The activity of mTORC1 is limited if serine863 remains not phosphorylated. After mTOR is on the active mode, mTOR can modulate Raptor activity that can be blocked by rapamycin [9].

H. Interaction between mTOR and MLST8

The fact of mTOR binding or binding with mLST8 is proven by [9] and Software String analysis in Fig. 8 with a binding score of 0.978. In the opposite, mLST8 supports mTOR kinase activity via the p70S6K and eIF4E-binding protein-binding 1 (4EBP1) initiation factors that bind to Raptor [9].

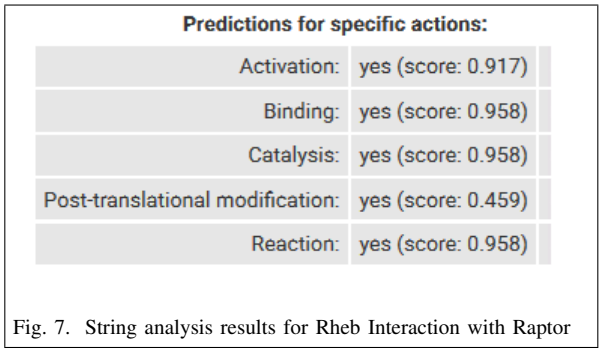

Fig. 7. String analysis results for Rheb Interaction with Raptor

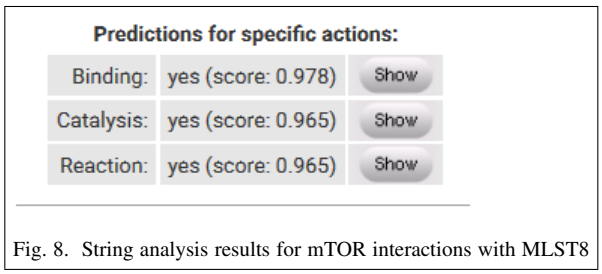

Fig. 8. String analysis results for mTOR interactions with MLST8

I. Interaction between Raptor and mTOR

The fact that Raptor binds to MTOR is proven by [15] and the String Software analysis with a binding score of 0.973. [15] mentions that mTOR is bound to Raptor. So, it can be concluded that there is a binding reaction between the two.

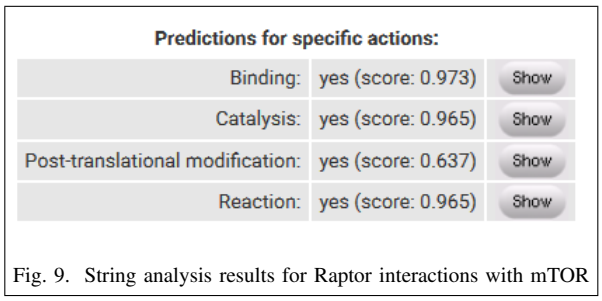

Fig. 9. String analysis results for Raptor interactions with mTOR

J. Interaction between mTORC1 with S6K

The fact that mTORC1 activates S6K1 by phosphorylating is proven by [16] and Software String analysis with an activation score of 0.964. mTORC1 will phosphorylate S6K1 substrate for normal cellular function [16]. So, it can be concluded that there is an interaction activation from mTORC1 to S6K1.

III. ARRANGING KINETIC REACTION ANALYSIS AND FORMULATING INTO ODE

Interactions among genes will be described in terms of kinetic reaction equations. The reaction is described in a different color. Green indicates that the gene acts as a reactant. Blue indicates the gene acts as a substrate. The red color indicates the gene acts as a complex of reactants and substrates. The pink color shows the product (reaction results) from the interaction of the two genes.

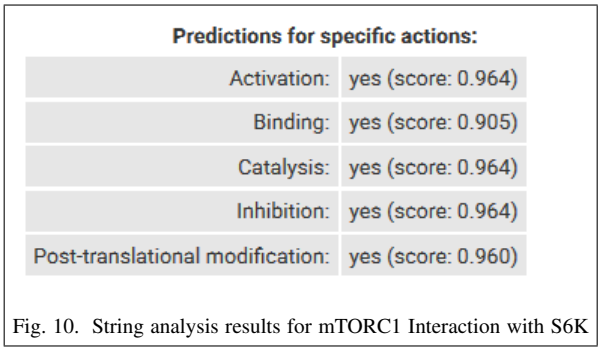

Fig. 10. String analysis results for mTORC1 Interaction with S6K

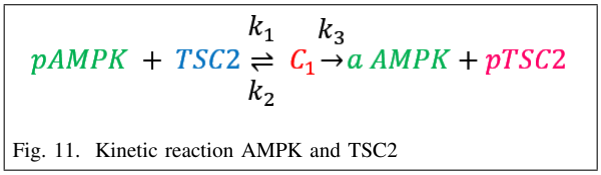

Fig. 11. Kinetic reaction AMPK and TSC2

A. AMPK and TSC2

When AMPK is active due to phosphorylation of AMPK-T172, it will inhibit mTORC1 by activating TSC2 through phosphorylation. From this, the reaction kinetics can be described in Figure 11, with aAMPK represents activated AMPK, pTSC2 represents phosphorylated TSC2. The reaction kinetics are formulated in the following ODE.

$$\frac{d[TSC2]}{dt} = k_2[C_1] - k_1[pAMPK][TSC2] \quad (1)$$

$$\frac{d[pAMPK]}{dt} = (k_2 + k_3)\,[C_1] - k_1[pAMPK][TSC2] \quad (2)$$

$$\frac{d[C_1]}{dt} = k_1[pAMPK][TSC2] - (k_2 + k_3)\,[C_1] \quad (3)$$

at the equilibrium point, the concentration rate does not change $\left(\frac{d[C_i]}{dt} = 0\right)$, so that

$$[C_1] = \frac{[pAMPK][TSC2]}{[TSC2] + \frac{k_1+k_3}{k_1}} \quad (4)$$

B. TSC2 and Rheb

After TSC2 is phosphorylated, there is an increasing of GAP activity. As a result, Rheb-GTP changes to Rheb-GDP, and mTORC1 activity is inhibited. Thus the reaction of pTSC2 with Rheb can be formed as follows. and from the reaction

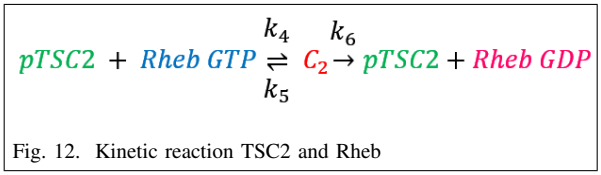

Fig. 12. Kinetic reaction TSC2 and Rheb

kinetics, we get

$$\frac{d[pTSC2]}{dt} = k_3[C_1] + (k_5 + k_6)\,[C_2] - k_4[pTSC2][Rheb^{GTP}] \quad (5)$$

$$\frac{d[Rheb^{GDP}]}{dt} = k_6[C_2] \quad (6)$$

$$\frac{d[C_2]}{dt} = k_4[pTSC2][Rheb^{GTP}] - (k_5 + k_6)[C_2] \quad (7)$$

as previous computation of $[C_1]$, we get $[C_2]$ below.

$$[C_2] = \frac{[pTSC2][Rheb]}{\frac{k_5+k_6}{k_4}} \quad (8)$$

(9)

Activated Rheb GDP can inhibit the process of protein synthesis. Therefore, GEF is needed to convert Rheb GDP to Rheb GTP. GEF for Rheb GDP is symbolized in Z(GEF), the interaction scheme is obtained below

$$Z(GEF) + Rheb\ GDP \underset{k_8}{\overset{k_7}{\rightleftharpoons}} C_3 \overset{k_9}{\rightarrow} Z(GEF) + Rheb\ GTP$$

Fig. 13. Kinetic reaction Z(GEF) and Rheb GDP

C. Rheb and mTORC1

Rheb GTP increases the activity of mTORC1, so it can be described by the following interaction scheme. and from the

$$Rheb\ GTP + mTOR \underset{k_{11}}{\overset{k_{10}}{\rightleftharpoons}} C_4 \overset{k_{12}}{\rightarrow} Rheb\ GTP + pmTOR$$

Fig. 14. Kinetic reaction Rheb GTP and mTORC1

reaction kinetics, we get

$$\frac{d[Rheb^{GTP}]}{dt} = (k_{11} + k_{12})[C_4] + (k_{17} + k_{18})[C_6] - k_{10}[Rheb^{GTP}][mTORC1] - k_{16}[Rheb^{GTP}][Raptor] \quad (10)$$

$$\frac{d[C_4]}{dt} = k_{10}[Rheb^{GTP}][mTORC1] - (k_{11} + k_{12})[C_4] \quad (11)$$

so

$$[C_4] = \frac{[mTOR]\left([Rheb^{GTP}_{tot}] - \frac{[Raptor][Rheb^{GTP}_{tot}]}{\frac{k_{17}+k_{18}}{k_{16}}+[Raptor]}\right)}{\frac{k_{11}+k_{12}}{k_{10}} + [mTOR] - \frac{[Raptor][Rheb^{GTP}_{tot}]}{\frac{k_{17}+k_{18}}{k_{16}}+[Raptor]}} \quad (12)$$

D. Rheb GTP and Raptor

Rheb GTP phosphorylates Raptor, so the following kinetic reaction is obtained:

$$\frac{d[Raptor]}{dt} = k_{17}[C_6] - k_{16}[Raptor][Rheb^{GTP}] + k_{24}[C_9] - k_{23}[PRAS40][Raptor] + k_{28}[C_{11}] - k_{29}[Raptor][mTOR] \quad (13)$$

$$\frac{d[C_6]}{dt} = k_{16}[Raptor][Rheb^{GTP}]$$

$$Rheb\ GTP + Raptor \underset{k_{17}}{\overset{k_{16}}{\rightleftharpoons}} C_6 \overset{k_{18}}{\rightarrow} Rheb\ GTP + pRaptor$$

Fig. 15. Kinetic reaction Rheb GTP and Raptor

$$- (k_{17} + k_{18})[C_6], \quad (14)$$

so

$$[C_6] = \frac{[Raptor]\left([Rheb^{GTP}_{tot}] - \frac{[mTOR]\left([Rheb^{GTP}_{tot}] - \frac{[Raptor][Rheb^{GTP}_{tot}]}{\frac{k_{17}+k_{18}}{k_{16}}+[Raptor]}\right)}{\frac{k_{11}+k_{12}}{k_{10}}+[mTOR]-\frac{[Raptor][Rheb^{GTP}_{tot}]}{\frac{k_{17}+k_{18}}{k_{16}}+[Raptor]}}\right)}{\frac{k_{17}+k_{18}}{k_{16}} + [Raptor]} \quad (15)$$

E. amTORC1 and S6K1

There is an activation reaction from mTORC1 to S6K1, so the following interaction scheme can be formed.

$$amTORC1 + S6K1 \underset{k_{14}}{\overset{k_{13}}{\rightleftharpoons}} C_5 \overset{k_{15}}{\rightarrow} amTORC1 + pS6K1$$

Fig. 16. Kinetic reaction amTORC1 and S6K1

$$\frac{d[S6KI]}{dt} = k_{14}[C_5]] - k_{13}[amTORC1][S6KI] \quad (16)$$

$$\frac{d[C_5]}{dt} = k_{13}[amTORC1][S6KI] - (k_{14} + k_{15})[C_5] \quad (17)$$

$$\frac{d[pS6KI]}{dt} = k_{15}[C_5]], \quad (18)$$

and we get $[C_6]$ as

$$[C_5] = \frac{[pmTOR][S6KI]}{\frac{k_{14}+k_{15}}{k_{13}}} \quad (19)$$

F. Deptor and mTOR

From Figure 6, Detor inhibits mTOR, so the kinetic reaction Deptor and mTOR shown below.

$$Deptor + mTOR \underset{k_{20}}{\overset{k_{19}}{\rightleftharpoons}} C_7$$

Fig. 17. Kinetic reaction Deptor and mTOR

$$\frac{d[Deptor]}{dt} = k_{20}[C_7] - k_{19}[Deptor][mTOR] \quad (20)$$

$$\frac{d[C_7]}{dt} = k_{19}[Deptor][mTOR] - k_{20}[C_7] \quad (21)$$

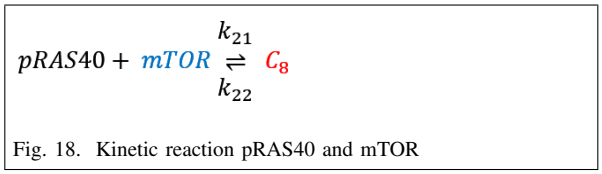

Fig. 18. Kinetic reaction pRAS40 and mTOR

G. pRAS40 and mTOR

PRAS40 can inhibit mTOR. The interaction of PRAS40 and mTOR did not produce any reaction products nor had any effect on increasing the activity of mTOR kinases.

$$\frac{d[PRAS40]}{dt} = k_{22}[C_8] - k_{21}[RAS40][mTOR] + k_{24}[C_9] - k_{23}[PRAS40][Raptor] \quad (22)$$

$$\frac{d[C_8]}{dt} = k_{21}[PRAS40][mTOR] - k_{22}[C_8] \quad (23)$$

H. pRAS40 and Raptor

PRAS40 also can inhibit Raptor.

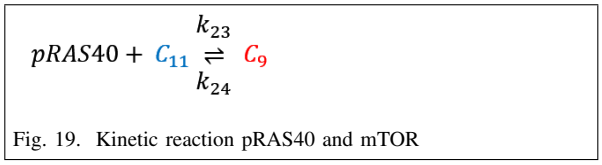

Fig. 19. Kinetic reaction pRAS40 and mTOR

I. mLST8 and mTOR

mLST8 increases mTOR activity, and the result of the interaction is amTORC1.

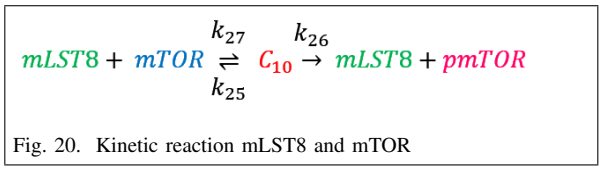

Fig. 20. Kinetic reaction mLST8 and mTOR

$$\frac{d[mLST8]}{dt} = k_{25}[C_{10}] - k_{27}[mLST8][mTOR] \quad (24)$$

$$\frac{d[C_9]}{dt} = k_{23}[PRAS40][Raptor] - k_{24}[C_9] \quad (25)$$

$$\frac{d[C_{10}]}{dt} = k_{27}[mLST8][mTOR] - k_{25}[C_{10}] \quad (26)$$

IV. CONCLUSION

In summary, mathematical modeling of protein mechanism Synthesis involving mTORC1 of the AMPK pathway is still in its infancy with many conceptual and technical challenges. So far, despite extensive biochemical knowledge of mTOR signaling, there has yet to be much successful mathematical modeling. New mechanical and logical models are needed to gain a complete understanding of the mTOR signaling pathway. Therefore, the specific contribution of mTOR signaling to aging, other signaling pathways, and appropriate targets for medication intervention. Therefore, computational modeling will continue to play an essential role as experimental research uncovers new mechanistic insights and addresses further questions related to mTOR signaling.

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