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Positive feedback in the Akt/mTOR pathway and its implications for growth signal progression in skeletal muscle cells: An analytical study

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ABSTRACT

The IGF-1 mediated Akt/mTOR pathway has been recently proposed as mediator of skeletal muscle growth and a positive feedback between Akt and mTOR was suggested to induce homogeneous growth signals along the whole spatial extension of such long cells. Here we develop two biologically justified approximations which we study under the presence of four different initial conditions that describe different paradigms of IGF-1 receptor-induced Akt/mTOR activation. In first scenario the activation of the feedback cascade was assumed to be mild or protein turnover considered to be high. In turn, in the second scenario the transcriptional regulation was assumed to maintain defined levels of inactive pro-enzymes. For both scenarios, we were able to obtain closed-form formulas for growth signal progression in time and space and found that a localised initial signal maintains its Gaussian shape, but gets delocalised and exponentially degraded. Importantly, mathematical treatment of the reaction diffusion system revealed that diffusion filtered out high frequencies of spatially periodic initiator signals suggesting that the muscle cell is robust against fluctuations in spatial receptor expression or activation. However, neither scenario was consistent with the presence of stably travelling signal waves. Our study highlights the role of feedback loops in spatiotemporal signal progression and results can be applied to studies in cell proliferation, cell differentiation and cell death in other spatially extended cells.

1. Introduction

The insulin-like growth factor (IGF-1) receptor pathway is a canonical pathway that mediates cell growth and survival. Upon growth factor binding to the receptor, the lipid kinase phosphoinositide-3-OH kinase (PI3K) gets phosphorylated and activated. This activation leads to phosphorylation and activation of the pro-survival kinase Akt. Through a double inhibitory step, active Akt leads to the activation of the mammalian target of rapamycin (mTOR) which switches on anabolic processes such as protein or nucleotide production. In turn, evidence has been provided that mTOR can phosphorylate and activate Akt (Granville et al., 2006; Sarbassov et al., 2005), thereby establishing a positive feedback loop.

Recent reports suggested the IGF-1 mediated PI3K-/Akt-/mTOR pathway to be a regulator of muscle cell growth. Indeed, studies that overexpressed active Akt by genetic mutations or pharmacological activation reported an increase in muscle fibre diameter (muscular hypertrophy) while muscle fibre diameter decreased upon inhibition of Akt or mTOR (muscular atrophy) (Pallafacchina et al., 2002; Bodine et al., 2001; Rommel et al., 2001). Trophic factor receptors such as IGF-1 can be randomly and anisotropically distributed along the cell surface (Grant et al., 1996; Kaiser et al., 1993; Wilkins et al., 2001) and activation of Akt by PI3K has been determined to be localised at the receptors. Therefore it remains to be understood how a signal that is associated with certain locations on the surface of a muscle cell can proceed through the entire spatial extension of this cell in a homogeneous fashion. Diffusion alone cannot be responsible for eliminating spatial gradients in larger cells as protein motility is limited by ubiquitenation and degradation of activated proteins (Huber et al., 2010). It is therefore assumed that the Akt/mTOR positive feedback loop acts as a signal regenerator (Ferrell, 2002; Kuroda et al., 2001; Tanaka and Augustine, 2008) in larger cells.

In this study, we will investigate a mathematical model that studies Akt/mTOR signal progression in skeletal muscle cells with an anisotropic distribution of IGF-1 receptors on the muscle cell surface. We will focus on an analytical treatment to study whether general principles of the resulting reaction diffusion equation can be derived. We will first discuss the time dependent, spatially invariant reaction system of a simple auto-activation network of Akt/mTOR and obtain criteria for stable enzyme

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activation. Subsequently, we will include one spatial dimension, so as to idealize the muscle cell as a linearly extended compartment and focus on two biologically reasonable scenarios that we solve in the presence of four different initiator signals. While no travelling waves are present under these assumptions, we will observe that the feedback loop together with diffusion can filter out spatial anisotropies. This filtering allows the translation of anisotropies arising from an inhomogeneous distribution of IGF-receptor or from fluctuations in the initiator signals into a homogeneous Akt/mTOR activation along the cell. We will further discuss the implications of our findings on receptor mediated activation in other large cells such as nerve cells.

2. Temporal dynamics of the Akt/mTOR auto-activation network

As we strive to obtain analytical solutions, we keep our system simple and represent it as a motif that is ubiquitously present in signalling pathways (see for example Huber et al., 2010). We concentrate on a time dependent analysis of the positive feedback loop to better characterise the basic feature of our reaction cascade. Instead of investigating a mutual activation between Akt and mTOR, we abstract the entire Akt/mTOR cassette into single node which activates itself (Fig. 1). This assumption is justified when mTOR levels vary more slowly than Akt, or the pool of mTOR is abundant in comparison to that of Akt, as shown in Section A.1. We further include a potential inhibitor of this Akt/mTOR node, which could be an Akt pharmacological antagonist, the Akt inhibitor phospholipid phosphatase SHIP-2 (Taylor et al., 2000), or the tumour suppressor

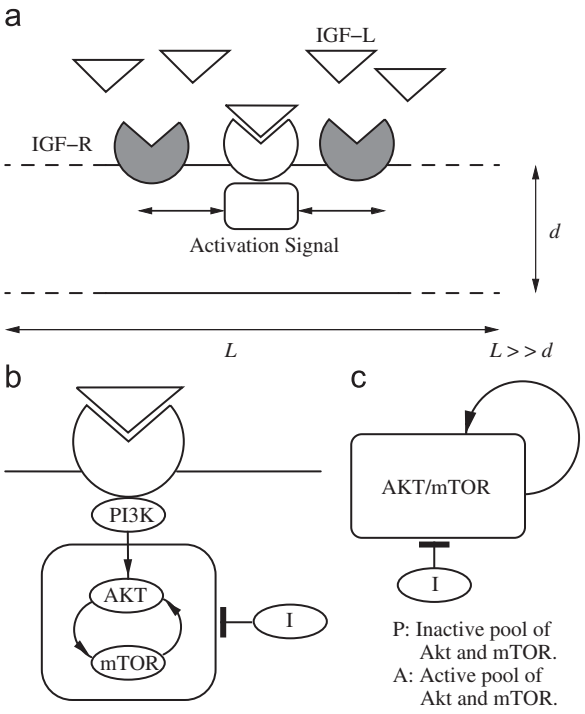

Fig. 1. Akt/mTOR pathway activation by IGF stimulation. The panel (a) shows a spatially extended cell ($L \gg d$), with IGF receptors (IGF-R) that are activated by binding of IGF ligands (IGF-L). Upon binding, the active signal that originates at the receptor propagates through the cell. The diameter d of the cell is neglected. The behaviour of the active signal at each spatial location is analysed in the main text. Panel (b) details how the receptors activation results in PI3 kinase activation and a subsequent positive feedback loop of Akt and mTOR. Moreover, the effect of an inhibitor I of such a pathway is considered. Panel (c) illustrates the abstraction of Akt and mTOR into one simple node which auto-activates itself as considered in this study. This auto-feedback is assumed to convert inactive versions of Akt/mTOR (P) to their respective active forms (A).

kinase protease and tensin homolog (PTEN) (Li et al., 1997; Manning and Cantley, 2007). Moreover, we assume that the Akt/mTOR form gets produced as inactive pro-form (P) and gets degraded when it is active (A). Only the active form is assumed to effect downstream signalling (Manning and Cantley, 2007). The resulting network is defined by

$$P + A \xrightarrow{k_1} 2A, \tag{1a}$$

$$A \xrightarrow{k_2} 0, \tag{1b}$$

$$P \underset{k_{3b}}{\overset{k_{3f}}{\rightleftharpoons}} 0, \tag{1c}$$

$$A + I \xrightarrow{k_4} 0, \tag{1d}$$

$$I \underset{k_{5b}}{\overset{k_{5f}}{\rightleftharpoons}} 0. \tag{1e}$$

Upon the presence of an active form A, the inactive pro-form P gets activated (auto-activation (1a)). In the kinetics of the auto-activation (1a), k_1 is set proportional to the product of the amount of the active and of the inactive form (consistent with the assumption of mass-action kinetics). The active form is assumed to get degraded (1b) and this degradation rate is assumed to be proportional to its concentration (factor k_2). Protein turnover was modelled to establish the pro-form P and the inhibitor I at constant expression levels (1c) and (1e), respectively. This was achieved by balancing protein production (linear factors k_{3b} and k_{5b} for inactive form and inhibitor) with protein degradation (first order proportionality constants k_{3f} and k_{5f}). Inhibition of the active form is assumed according to mass action kinetics (1d).

Defining the vector $\mathbf{c} = (c_1\ c_2\ c_3)^T := ([P]\ [A]\ [I])^T \in \mathbb{R}^3_+$, we obtain the following set of coupled ordinary differential equations (ODEs) (see Klipp et al., 2005, for details)

$$\dot{c}_1 = -k_{3f}c_1 - k_1c_1c_2 + k_{3b}, \tag{2a}$$

$$\dot{c}_2 = -k_2c_2 + k_1c_1c_2 - k_4c_2c_3, \tag{2b}$$

$$\dot{c}_3 = -k_{5f}c_3 - k_4c_2c_3 + k_{5b}. \tag{2c}$$

Without the inhibitor, we obtain

$$\dot{c}_1 = -k_{3f}c_1 - k_1c_1c_2 + k_{3b}, \tag{3a}$$

$$\dot{c}_2 = -k_2c_2 + k_1c_1c_2. \tag{3b}$$

Due to the nonlinearity of this equation, a straightforward mathematical solution of the temporal dynamics is not possible. However, we can calculate the steady state protein levels for the inactive form ($\bar{c}_1$) and the active form ($\bar{c}_2$) in the auto-activation cascade,

$$\text{off state}: \ \{\bar{c}_{1,off}, \bar{c}_{2,off}\} = \left\{\frac{k_{3b}}{k_{3f}}, 0\right\}, \tag{4a}$$

$$\text{on state}: \ \{\bar{c}_{1,on}, \bar{c}_{2,on}\} = \left\{\frac{k_2}{k_1}, \frac{k_{3b}}{k_2} - \frac{k_{3f}}{k_1}\right\}. \tag{4b}$$

Here, we have denoted the state where no active form is present as the 'off state' while the other state was denoted as 'on state'. We next investigated under what conditions these steady states are stable against small perturbation. Such a local stability analysis has been extensively described by Eissing et al. (2004). In brief, local stability requires that the eigenvalues of the Jacobian matrix that were derived from the system (2a)–(2c) have negative real parts. The eigenvalues are roots of the