Published in final edited form as:

Methods Mol Biol. 2019 ; 1890: 205–217. doi:10.1007/978-1-4939-8900-3_17.

Mathematical Modeling of Nuclear Trafficking of FOXO Transcription Factors

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Abstract

Nuclear cytoplasmic flux of Foxo transcription factors is paramount in cellular gene regulation. For example, excluding Foxo from skeletal muscle nuclei is necessary to avoid muscle wasting through elevated protein breakdown. Constructing a mathematical model of the signaling process leading to alteration of Foxo nuclear cytoplasm ratio is useful in predicting and interpreting such ratio changes. In this chapter we derive a general mathematical model for nuclear cytoplasmic flux. We apply this model to Foxo flux and take advantage of rapid phosphorylation approximation and conservation conditions to reduce the Foxo flux model. We constrain our model with data from mouse skeletal muscle with applied IGF. This procedure provides an example of what might be called the central approach of mathematical modeling: The cycling of a biological question through mathematical formulation and back to biological interpretation.

Keywords

Mathematical modeling; Nuclear cytoplasmic flux; Foxo transcription factor; Skeletal muscle

1 Introduction

Mathematical modeling provides a way to translate biological old pathways to results that, upon conversion back in to the biological context, may enlighten that which might otherwise remain hidden. Here, we consider mathematical modeling as applied to nucleo/cytoplasmic movements of Foxo1, a member of the DNA-binding forkhead domain class of transcription factors, in living cells.

The general process follows a cycle: (1) Formulation of a biological question; (2) Articulation of the related biological process as elementary steps; (3) Translation of elementary steps into a mathematical formulation; (4) Analysis and Simulation of the formulated model possibly including time course, parameter variation mimicking experiment, parameter estimation based on experiment, sensitivity to these parameters; (5) Interpretation of mathematical results in the biological context; (6) Repeat, but once the core model is established going from questions (1) to analysis (4) is often much more efficient.

In the present chapter we detail steps applied to study nucleo/cytoplasmic translocation of Foxo-1. Details of Foxo background as needed to motivate the question(s) and set up the steps can be found in other chapters.

Specifically for Foxo we can ask, “How does Foxo translocate into and out of the nucleus in response to external signals?” In general the full system of Foxo posttranslational modifications (PTMs; e.g., phosphorylation, methylation, acetylation, ubiquitination) is complex. However, individual modifications can be selectively examined by modulating individual PTM pathways using specific biological or pharmacological ligands, while leaving pathways to other PTMs relatively unperturbed [1–4]. So the focus we take is on a critical component, phosphorylation by Akt at a single effectively lumped cite. This works well with the experimental data of nuclear and cytoplasmic fluorescence of the fusion protein of Foxo1 with green fluorescent protein (Foxo1-GFP).

We construct a mathematical model using several applied mathematics techniques such as mass balance, law of mass action, conservation, and volume ratios, which helps to simplify systems [5]. Specifically, we observe reductions due to large cytoplasmic-nuclear volume ratio and the assumption that de/phosphorylation is relatively fast compared to nuclear flux. These reductions are implemented in a reduced model. Furthermore, assumptions are made of negligible protein synthesis or breakdown over a 1–2 h experiment. We can then analyze the system solving it analytically, where possible, or numerically, observe the time course of the nucleo/cytoplasmic ratio in control and various experimental conditions (e.g., applications of pathway modifiers AKT-I, PI3K-I, IGF-1, etc.), and test the parameters and their sensitivities. Critically the analysis must be interpreted in the biological context and compared to data. Inevitably shortcomings are revealed or new ideas are formed that must be addressed.

We develop these techniques in terms of a general case in Subheading 2.1, and then apply them specifically to Foxo1 in Subheading 2.2.

1.1 Modeling Introduction

Modeling transcription factor action can have multiple facets from posttranslational modifications to transfer between cytosolic and nuclear spaces to DNA-binding affinities. However, the components used to model such a system should be chosen to accommodate comparisons with data that may be available. Model predictions may then dictate other key data that should be obtained in subsequent experiments. A schematic of fluxes and interactions for transcription factors is shown in Fig. 1.

We describe in general three mathematical modeling techniques that apply to the Foxo system with current measurement methods. (1) The Law of Mass Action provides a way to track the rate of change of a reacting chemical population including flux balance as a system of ordinary differential equations (ODEs). (2) Using that the relative volume fraction of the nucleus is quite small compared to the cytosolic volume in muscle cells, the system of ODEs can be reduced using a small perturbation approximation. (3) Parameter estimation in comparison with related data generates a verified model.

With a verified model system, we test the system by predicting outcomes of novel experiments. The results of these experiments then indicate the validity or shortcomings of the model, which may then be revised.

1.2 Introduction

Using the general approach, we will create a mathematical model that investigates the nucleo cytoplasmic movements of Foxo1 inside the skeletal muscle fiber when external stimuli are applied.

The transcription factor Foxo1 has specific action in skeletal muscle for regulation of muscle mass. Foxo1 translocation from the cytosol into the nucleus initiates transcription of factors that upregulate proteins that lead to muscle protein degradation and consequent muscle atrophy. The wider scope of this project is to gain insight into which, if any, external stimuli or pharmacological agents could potentially reduce the rate of skeletal muscle atrophy. This insight would help patients who are on bed rest because of aging or a medical ailment because their recovery process would become easier if their muscles were not as weak as they become when immobile.

2 Methods

2.1 Mathematical Modeling

We begin by describing the mathematical methods in the general context of Fig. 1.

2.1.1 Reaction—We use the Michaelis-Menten formalism to write the enzymatic conversion, $x \rightarrow y$, of one molecule (concentration = x) into another molecule (concentration = y), such as that in phosphorylation, by $dx/dt = -k_{cat} \times E \times x / (x + K_m) = -dy/dt$, where k_{cat} is the catalysis step rate, E is the concentration of enzyme, and K_m is the backward enzyme binding plus catalysis rates divided by the forward enzyme-binding rate, $K_m = (k_b + k_{cat})/k_f$. If the enzymatic process is well below saturation then we can assume that x is much less than K_m and so $dx/dt \sim -k_{cat} \times E/K_m \times x = -kp \times x$. And the loss of x leads to the gain in y , so $dy/dt = kp \times x$. Similarly, in the reverse reaction, $y \rightarrow x$, can result in the loss of y , $dy/dt = -ku \times y$ and the gain of x , $dx/dt = ku \times y$. Combining both of these processes yields for x , $dx/dt = -kp \times x + ku \times y$. This reaction can occur both in the cytoplasm (involving xc and yc), and in the nuclei (involving xn and yn) (see Note 1).

2.1.2 Translocation—Furthermore, we can think of the movement from one space into another as either diffusion (i.e., without active transport) or actively transported. The rate of change of say xn under diffusion would just be proportional to the difference in concentrations across the boundary, $dxn/dt = D(xc - xn)$. However, an active process

¹If enzyme behavior, instead of near its sensitivity (K_m), is near saturating ($x \gg K_m$), then.

$$dx/dt = -k_{cat} \times E,$$

a constant degradation rate. But this approximation can become problematic if x does decay and $x \gg K_m$ becomes inappropriate.

assuming sufficient energy would only depend upon the driving side concentration much like the enzymatic process, i.e., $dxn/dt = kI \times xc$ where kI is the influx rate constant.

For molecules larger than about 40 kD nuclear influx and efflux both occur via corresponding nuclear import or nuclear export machinery that requires concentration gradients of co-transported molecules to be present. We assume that the concentrations of these co-transported molecules remain constant during an experiment. We further assume that the transported molecules (xc or yn) are at sufficiently low concentration that their unidirectional flux is proportional to their concentration (i.e., the transport systems are far from saturation by x or y , and are thus operating in their linear range in terms of x or y).

In response to transport, the molecules in the original space are decremented, $dxn/dt = -kI \times xc$. Similarly for efflux, $dyc/dt = kE \times yn$ and $dyn/dt = -kE \times yn$. Now this is true if the two spaces are equal in size. However, if they are not of equal size, molecular balance must be taken into account.

2.1.3 Impact of Disparate Volume Fractions—To account for volumes, say V , that might be different we write concentrations x and y as number of molecules divided by volume, $x = X/V$ and $y = Y/V$. For reactions in the same space, using molecules or concentrations is equivalent but for reactions crossing spaces, such as the influx and efflux, we need to write the balance in terms of molecules $dXn/dt = kI \times Xc$. We would like to have the quantities in terms of concentrations however, so we multiply and divide by (constant nuclear and cytosolic, for example) (see Note 2) volumes, V_n and V_c , to get.

$$V_n \times d(X_n/V_n)/dt = V_c \times kI \times Xc/V_c$$

or $dxn/dt = kI \times V_c/V_n \times xc$. From this we can see an important point that the rate of change of the number of molecules in one space can be greatly affected by the volume ratio of the spaces. Similarly, the efflux can be represented as $dyc/dt = kE \times V_n/V_c \times yn$.

2.1.4 Combining Processes—Combining the reactions within each space and fluxes between spaces as drawn in Fig. 1 we get

$$dxn/dt = -kpn^*xn + kun^*yn + kl^*Vc/Vn^*xc$$

$$dyn/dt = kpn^*xn - kun^*yn - kE^*yn$$

².If we are concerned about volumes changing in time, then we should write X as $x \times V$, so that.

$$dX/dt = d(x \times V)/dt = dx/dt \times V + x \times dV/dt$$

The additional term, $x \times dV/dt$ scaled by the volume, would have to be subtracted from the right hand side of the equation for dx/dt showing that if the volume is increasing for any level of x this effectively decreases the concentration.

$$dxc/dt = -kpn^*xc + kun^*yc - kl^*xc$$

$$dyc/dt = kpn^*xc + kun^*yc + kE^*Vn/Vc^*yn,$$

where influx, efflux, or reaction terms are color coded as in Fig. 1.

2.2 Application of Modeling to Foxo

We now apply the mathematical techniques described in the previous section to Foxo flux between cytosol and nucleus.

2.2.1 Phosphorylation-Dependent Foxo Nucleo-Cytoplasmic Fluxes—To enter the nucleus Foxo1 must be unphosphorylated. Foxo is phosphorylated in response to any of a number of upstream factors that activate Akt [6, 7], and is dephosphorylated by protein phosphatase 2A [8].

Akt is traditionally located in the cytosol. However, phosphorylated Foxo1 is extruded from the nucleus after entering unphosphorylated indicating a phosphorylation mechanism within the nucleus as well as the cytosol. Measuring effective influx and efflux rates of unphosphorylated and phosphorylated Foxo, respectively informs the mechanisms of phosphorylation and flux, but these confounding processes cannot be isolated using current measurement methods. Mathematical methods may prove useful for quantifying these processes if not assisting to disaggregate them. Specifically, what is the change in influx and efflux in the presence of external stimuli?

Figure 2a shows a cartoon of the known processes that occur within the skeletal muscle cell. The application of an external stimulus, such as insulin growth factor 1 (IGF-1), sets off a domino series of events leading up to the phosphorylation of the Forkhead Box transcription factor1 (Foxo-1) in the nucleus and in the cytoplasm. It is important to recognize that only unphosphorylated Foxo-1 can translocate into the nucleus and phosphorylated Foxo-1 can translocate into the cytoplasm. A higher proportion of Foxo-1 in the nucleus leads to a faster rate of skeletal muscle protein degradation due to Foxo activation of transcription of ubiquitin ligases [9].

Because of this fact, we aim to examine the nuclear to cytosolic ratio of Foxo-1 in order to determine if various external stimuli will cause muscle degradation or prevent it.

2.2.2 Four State Model—We create a four-state model, as shown in Fig. 2b(A), to describe the flow between phosphorylated and unphosphorylated states in both the nucleus and cytosol as well as between them. Using the laws of mass action, we translate the diagram into a set of equations to describe this flow, shown in equation set Eq. (1). Looking specifically at the creation of the rate of change for dephosphorylation in the cytoplasm, $\frac{dU_c}{dt}$. Figure 2b(A) shows that dephosphorylated Foxo-1 is gained through the rate of dephosphorylation of Foxo in the cytoplasm from phosphorylated Foxo-1 in the cytoplasm

($k_{U_c}P_c$), phosphorylated Foxo-1 is lost through the rate of phosphorylation in the cytoplasm from dephosphorylated Foxo-1 in the cytoplasm ($-k_{P_c}U_c$), and dephosphorylated Foxo-1 is lost through the rate of influx into the nucleus from dephosphorylated Foxo-1 in the cytoplasm ($-k_IU_c$). Summing these three components results in $\frac{dU_c}{dt}$ equation set (1). The same method can be applied for the remaining three equations in equation set (1).

$$\begin{aligned}\frac{dU_c}{dt} &= -k_{P_c}U_c + k_{U_c}P_c - k_IU_c \\ \frac{dP_c}{dt} &= k_{P_c}U_c - k_{U_c}P_c + \frac{V_n}{V_c}k_E P_n \\ \frac{dU_n}{dt} &= -k_{P_n}U_n + k_{U_n}P_n + \frac{V_c}{V_n}k_IU_c \\ \frac{dP_n}{dt} &= k_{P_n}U_n - k_{U_n}P_n - k_E P_n\end{aligned}\quad (1)$$

where k_{P_n} is the rate constant of nuclear phosphorylation, k_{U_n} is the rate constant of nuclear dephosphorylation, k_{P_c} is the rate constant of cytosolic phosphorylation, k_{U_c} is the rate constant of cytosolic dephosphorylation, k_I is the rate constant of Foxo-1 influx into the nucleus, k_E is the rate constant of efflux from the nucleus, V_c is the cytosolic volume, and V_n is the nuclear volume [10].

We can sum concentration of phosphorylated and unphosphorylated Foxo-1 in the cytoplasm and in the nucleus, respectively,

$$\begin{aligned}C &= U_c + P_c, \\ N &= U_n + P_n,\end{aligned}$$

where C is the total concentration (phosphorylated plus unphosphorylated) of Foxo-1 in the cytoplasm and N is the total concentration (phosphorylated plus unphosphorylated) of Foxo-1 in the nucleus. Similarly, we can take the time derivatives of C and N to get:

$$\begin{aligned}\frac{dC}{dt} &= \frac{dU_c}{dt} + \frac{dP_c}{dt} = \hat{k}_E P_n - k_I U_c \\ \frac{dN}{dt} &= \frac{dU_n}{dt} + \frac{dP_n}{dt} = -\frac{V_c}{V_n} \hat{k}_E P_n + \frac{V_c}{V_n} k_I U_c\end{aligned}$$

where $\hat{k}_E = \frac{V_n}{V_c} k_E$ is now the fixed scaled efflux.

We assume that the total number of molecules of Foxo1 in a cell (phosphorylated and dephosphorylated; in the nucleus and in the cytoplasm) to be conserved over the time of the experiments (i.e., there is negligible net synthesis or degradation of Foxo1 during an experiment). This can be seen as summing the two previous equations while scaling the nuclear equation by $\varepsilon = \frac{V_n}{V_c}$ to get $\frac{dC}{dt} + \varepsilon \frac{dN}{dt} = 0$ (see Note 3).

2.2.3 Rapid De/Phosphorylation Assumption Reduces Four-State Model—

Using a rapid phosphorylation and dephosphorylation approximation we can reduce a

system like that in Section A4 to a reduced system that is appropriate and more simply analyzed.

Assuming that the phosphorylation and dephosphorylation processes occur much faster than the rates of influx and efflux, we can then consider the rapid equilibration situation [10]. Accordingly, $k_{Pc}U_c = k_{Uc}P_c$ in the cytoplasm, and $k_{Pn}U_n = k_{Un}P_n$ in the nucleus. With these relations and the conservation equations, it is possible to produce the effective efflux (k'_E) and effective influx (k'_I) rate constants shown in Fig. 2b(B). These new rate constants are the previous influx and efflux rate constants, seen in Fig. 2b(A), scaled by rate constant fractions to yield equation set (2)

$$\begin{aligned}\frac{dC}{dt} &= \epsilon(k'_E N - k'_I C) \\ \frac{dN}{dt} &= -k'_E N + Dk'_I C\end{aligned}\quad (2)$$

Equation set (2) has modified rate constants in order to absorb the phosphorylation and dephosphorylation rate constants in the four-state model. These modified rate constants are described in equation set (3).

$$\begin{aligned}k'_I &= \frac{k_{Uc}}{k_{Uc} + k_{Pc}} k_I \\ k'_E &= \frac{k_{Pn}}{k_{Pn} + k_{Un}} \hat{k}_E\end{aligned}\quad (3)$$

Equations (3) in represent the effective fluxes in the two-state model in Fig. 2b(B). If we rewrite these equations we can see these as the fraction of dephosphorylation in the cytoplasm or the fraction of phosphorylation in the nucleus, respectively

$$\begin{aligned}\frac{k'_I}{k_I} &= \frac{k_{Uc}}{k_{Uc} + k_{Pc}} \\ \frac{k'_E}{\hat{k}_E} &= \frac{k_{Pn}}{k_{Pn} + k_{Un}}\end{aligned}$$

When $k'_I \rightarrow k_I$ then the cell is approaching full cytoplasmic dephosphorylation and, similarly, when $k'_E \rightarrow \hat{k}_E$ the cell is approaching full nuclear phosphorylation.

2.2.4 Solution to the Reduced System—Part of the value to reducing the four-variable system is the ability to readily solve the system and interpret the terms in a biophysical way. Conservation of Foxo-1 in the entire system (nucleus and cytosol) gives us

$$3. \frac{dC}{dt} + \epsilon \frac{dN}{dt} = 0 \text{ is conservation of Foxo in the cell seen by integrating up the equation to get } C + \epsilon N = \text{Constant}$$

Recalling $\epsilon = \frac{V_n}{V_c}$, then $V_c \times C + V_n \times N = \# \text{ Foxo molecules in the cytoplasm and nucleus.}$

the relationship $C_T = C + \varepsilon N$ where C_T is the initial total amount of Foxo-1 in the Cytoplasm. We can use the integrating factor method of ordinary differential equations to solve for $N(t)$ from equation set (2).

$$N(t) = C_T \left[\left(\frac{N_0}{C_T} - \frac{k'_I}{k'_E + \varepsilon k'_I} \right) e^{-(k'_E + \varepsilon k'_I)t} + \frac{k'_I}{k'_E + \varepsilon k'_I} \right]$$

Now we can divide $N(t)$ by $C \sim C_T$ and using this conservation equation as well as taking the limit as epsilon goes to 0, both for the condition of negligible nuclear compared to cytoplasmic volume in muscle fibers [8] we obtain the following nuclear to cytosolic ratio of Foxo-1 as a function of time:

$$\frac{N}{C}(t) = \left[\left(\frac{N_0}{C_T} - \frac{k'_I}{k'_E} \right) e^{-(k'_E)t} + \frac{k'_I}{k'_E} \right] \quad (4)$$

where N_0/C_T is the initial nuclear/cytoplasmic ratio of Foxo-1, k'_I / k'_E is the final value of N/C , and k'_E is the rate constant for the change from the initial to final N/C (see Note 4).

An important aspect of this more or less straightforward exponential equation is that this model contains all of the constant rates of phosphorylation, influx, and efflux imbedded within composite parameters k'_E and k'_I , which are fitted using the experimental data and can be manipulated to accommodate and even predict results of new experiments.

2.2.5 Obtaining the Experimental Data—To achieve experimental monitoring of Foxo nucleo/cytoplasmic distribution adult mouse skeletal muscle fibers in culture serve as our primary model system for live cell time course confocal imaging. As in numerous previous studies from our lab, we will use a computer controlled high-performance confocal fluorescence microscope system to obtain fluorescence images of multiple cultured muscle fibers in parallel over a time period of 1–2 h, from which we determine the time course of the nuclear/cytoplasmic distribution of Foxo1-GFP. This model muscle fiber system provides an excellent experimental tool for studying molecular mechanisms for transcription factor signaling in live skeletal muscle fibers, as we do here with Foxo1-GFP.

In the image acquisition and analysis of our system, the time course of Foxo1-GFP can be tracked simultaneously in nuclei and cytoplasm in successive images of multiple fibers by using a computer controlled stage on a laser scanning confocal fluorescence microscope. Using a single four-compartment commercial cover glass bottom culture dish, fibers can be monitored in parallel under four different experimental conditions during the same experiment. Following the experiment, mean pixel fluorescence, proportional to mean Foxo1-GFP concentration, is determined in user-defined nuclear (N) and cytoplasmic (C) areas of interest (Fig. 3a) using image analysis software (e.g., Image J).

⁴.Notice that in Eq. (2) we could have used that C is not changing very rapidly to hold C constant, then dividing the N equation by C we get the scalar ordinary differential Eq. (4) $\frac{d(N/C)}{dt} = -k'_E(N/C) + k'_I$, which, of course, leads to the same solution in (4).

2.2.6 Model Fit to Experimental Data—Foxo1 is *not* at steady state at the start of the experimental period, but approaches steady state under control conditions. “Control” muscle fibers that were not exposed to any agent exhibit a relatively slow gradual rise in nuclear Foxo1-GFP (Fig. 3b), when the bathing solution is changed from culture medium to fresh growth factor-free recording solution about 30 to 45 min prior to the start of recording. Application of fresh medium causes the nuclear to cytoplasmic concentration ratio (N/C) of Foxo1-GFP in the fibers to increase toward a new quasi-steady state level characteristic of the growth factor-free experimental condition. N/C approaches its new steady state along a single-exponential time course (curve fit to data in Fig. 3b), as predicted by the reduced model. Using the reduced model (above), the single-exponential function fit to the time course of N/C under control conditions (Fig. 3b) yields values for the apparent unidirectional rate constants for Foxo1 nuclear influx and efflux, k'_I and k'_E respectively, under the control conditions.

IGF1 induces a marked *decrease* in the apparent unidirectional rate constant for Foxo1-GFP nuclear influx. Using Foxo1-GFP expressed in adult FDB muscle fibers, we first showed that IGF1 (100 ng/mL), which activates the PI3K/Akt pathway, caused a marked and rapid decline in N/C (Fig. 4a; recorded using 2 min sampling intervals between images in order to capture the rapid time course of decline of N/C , and presented on an expanded time scale compared to Fig. 3b). The observed rapid and pronounced decrease in nuclear Foxo1-GFP after IGF1 addition (Fig. 4a) could in principal be due to an increased unidirectional nuclear efflux, a decreased unidirectional influx, or some combination of the two. Without a model it is not apparent what the underlying change may be, but by applying the 2-state reduced mathematical model presented here to the measured Foxo1 nuclear cytoplasmic redistribution we first found that the model could closely reproduce the observed N/C time course under control conditions (curve fit to data in Fig. 3b) and during activation of Akt activity by IGF1 (curve fit to declining phase in Fig. 4a). The values for the apparent rate constant k'_I for unidirectional nuclear influx of Foxo1-GFP obtained from the fits were decreased by about an order of magnitude in IGF1 (Fig. 4b, k'_I , left filled bar compared to open bar control), indicative of a similarly *de*creased fractional *de*phosphorylation (i.e., increased phosphorylation) of Foxo1 in the cytoplasm, as expected for IGF1 activation of the IGF1/PI3K/Akt pathway in the cytoplasm.

IGF1 application also dramatically *increases* the apparent rate constant for FOXO1-GFP unidirectional nuclear efflux by over 8 times (Fig. 4b, k'_E , right filled bar compared to right open bar control), indicating an over eightfold increase in fractional phosphorylation of Foxo1 within the nuclei. This novel observation indicates that IGF1 strongly activates Akt (or some other Foxo1 kinase that promotes Foxo1 nuclear efflux) within the muscle fiber nuclei. This implies that a signaling pathway must be present to link the plasma membrane IGF1 receptor to the intra nuclear activity of Akt (or of some other intra nuclear Foxo phosphorylating enzyme). The decrease in k'_I and the increase in k'_E in response to IGF1 application produce a physiologically coordinated response to IGF1 since both changes have the effect of decreasing the nuclear content of Foxo1, thus decreasing Foxo1 transcriptional effectiveness. Prior to our studies there was little prior information regarding the time courses of Foxo1 nuclear movements, and almost no consideration of the relative roles of

nuclear vs. cytoplasmic signaling pathways and mechanisms regulating the nuclear vs. cytoplasmic changes.

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Fig. 1.
Core processes in posttranslational modification (PTM)-dependent nucleo-cytoplasmic translocation in a cell

Fig. 2.

(a) From Wimmer et al. [10], the cascade of events that occur within the skeletal muscle cell when an external stimuli is injected. Upon IGF-1 being injected, PI3K is activated, which in turn activates Akt to phosphorylate Foxo-1 in the cytoplasm beginning the translocation flow of Foxo-1 into and out of the nucleus. A similar pathway to Akt phosphorylates Foxo-1 in the nucleus, but it is not yet known what pathway this is. (b) (A) Schematic representing the four-state model of the atrophy process within the skeletal muscle cell. This model describes the flow of Foxo-1 through phosphorylation in the cytoplasm, unphosphorylation in the cytoplasm, phosphorylation in the nucleus, unphosphorylation in the nucleus, and influx and efflux into and out of the nucleus, respectively. (b) Effective influx and efflux of Foxo-1 after compressing the four-state model into a two-state model where the influx and efflux rates absorb the phosphorylation rates

Fig. 3.

(a) Fluorescence in skeletal muscle showing Foxo-GFP in the nuclei and cytoplasm. (b) Data and model fit to control N/C time course. Error bars are ± 1 SEM. For symbols lacking error bars, error is less than symbol size. Bar plot bars without error bars are from fits to the average time course

Fig. 4.
(a) Application of IGF rapidly decreases N/C (data and model fit). **(b)** Effective flux parameters estimated from the data. In the presence of IGF the influx decreases while the efflux is significantly increased