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Review

mTOR signalling, embryogenesis and the control of lung development

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ABSTRACT

The existence of a nutrient sensitive "autocatakinetic" regulator of embryonic tissue growth has been hypothesised since the early 20th century, beginning with pioneering work on the determinants of foetal size by the Australian physiologist, Thorburn Brailsford-Robertson. We now know that the mammalian target of rapamycin complexes (mTORC1 and 2) perform this essential function in all eukaryotic tissues by balancing nutrient and energy supply during the first stages of embryonic cleavage, the formation of embryonic stem cell layers and niches, the highly specified programmes of tissue growth during organogenesis and, at birth, paving the way for the first few breaths of life. This review provides a synopsis of the role of the mTOR complexes in each of these events, culminating in an analysis of lung branching morphogenesis as a way of demonstrating the central role mTOR in defining organ structural complexity. We conclude that the mTOR complexes satisfy the key requirements of a nutrient sensitive growth controller and can therefore be considered as Brailsford-Robertson's autocatakinetic centre that drives tissue growth programmes during foetal development.

Contents

1. Early understanding of growth and nutrient sensing processes in developmental biology

Joseph Needham's *Chemical Embryology* published in 1931 [1] gives a fascinating and exhaustive perspective of the history and cultural understanding of foetal development beginning with Ancient Egyptian methods of artificial bird egg incubation through to the rather lurid descriptions of foetal fluids and membranes,

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summarised in 1814 by J.F. John in his "*Chemische Tabellen des Tierreichs*". At the time Needham's book was published, much attention was given to defining a universal quantitative formula for the relationship between tissue size and foetal weight and Needham comprehensively covered this debate arriving at a rather conflicting set of conclusions:

i) Mitotic index falls with increasing age of the foetus, yet, within this constraint,
ii) Different cells of the body mature and slow at different rates. For all that, the gross pattern of foetal weight gain over time shows,
iii) S-shaped kinetics that is characteristic of an endogenous growth controller, a so-called "autocatakinetic" response.

This last view is probably the most interesting since it is the first acknowledgement that a critical control point governs the size of growing biological systems. The main proponent of this "autocatakinetic hypothesis" was Thorburn Brailsford-Robertson, a Scots-born Australian, who in 1908 argued that "*a master reaction. . .would act as the limiting factor of growth, and would impress its own particular character on the general appearance of the whole process from the outside*" [2]. With considerable foresight, he argues that this master reaction would likely be an endogenous cell regulator whose key characteristic being that it was nutrient sensitive and capable of directing the related processes of what we now know to be gene expression and protein synthesis, a concept dismissed by Needham as being "*definitely out of court*". It was therefore clear from the early 20th century that individual cells possessed some kind of nutrient-sensitive regulator of cell growth and differentiation; 83 years later, Heitman et al. [3] made the link between rapamycin, a macrolide inhibitor of cell growth produced by *Streptomyces hygroscopius*, and yeast kinases, target of rapamycin (TOR) 1 and 2 [4,5]. 4 years later a single mammalian homologue was identified which shared the nutrient and rapamycin sensitive properties of its yeast ancestors and was ultimately named mTOR [6–8]. Thus, although modern day understanding of eukaryotic TOR signalling originated from yeast biology, the philosophical origins of a nutrient sensitive "autocatakinetic" regulator of cell growth is firmly rooted in developmental biology and has a documented history that dates back 5000 years to the beginnings of Egyptian agrarian civilisation and mass food production. With this in mind, and as tribute to the foresight of Brailsford-Robertson and his predecessors, the purpose of this review is to identify the key roles played by mTOR during embryogenesis and development. By taking the lung branching morphogenesis programme as an example, we show how this kinase regulates the formation of complex organ structures by impressing "*its own character on the general appearance of the whole process from the outside.*" [1].

2. The initiation of protein synthesis and growth in the fertilised oocyte

The first moment of embryonic development begins with the controlled reversal of metaphase II growth arrest in oocytes in response to the acrosome reaction at the head of the sperm that enables gamete fusion and exocytosis of the sperm nucleus into the egg. In mammals, this event is coordinated by a series of calcium signalling events which begin with the interaction between the sperm head and an oocyte surface matrix glycoprotein, zona pellucida 3 (ZP3 [9]). ZP3 stimulates production of phosphatidylinositol-(3,4,5)-triphosphate in sperm membranes inducing downstream Akt signalling (a regulator of mTOR but unconfirmed in sperm) and release of phospholipase Cζ, leading to Ca^{2+} spikes in the oocyte which persist for hours after fertilisation. These Ca^{2+} oscillations are necessary and sufficient for orchestrating all fertilisation events including exit from metaphase II (MII) arrest and the initiation of cell cycle activity and so can be thought of as the cue that mediates the very first induction of transcription and translation. MII arrest is sustained in quiescent oocytes by activation of the cyclinB/cell division cycle protein 2 kinase (cdc2) heterodimer, collectively known as maturation promoting factor (MPF). This prolonged activity is unique to MII and is maintained by a cytostatic factor (CSF) known as early mitotic inhibitor 2 (Emi2) which prevents the targeted degradation of cyclinB by sequestering cdc20, an activator of the anaphase promoting complex/cyclosome (APCC) ubiquitin ligase (Fig. 1). On fertilisation, Ca^{2+} oscillations induce cyclic calmodulin kinase II (CamKII) activity which phosphorylates Emi2 at a RSST motif spanning amino acids 192-5, inducing a strong interaction with polo like kinase-1 (Plk1) leading to a secondary Emi2 phosphorylation within its N-terminal phosphodegron domain ($DSGX_nS$)[10,11]. In this configuration, Emi2 is ubiquitylated by SCF(βTrCP) and its clearance permits cdc20 to activate APCC and degrade cyclinB enabling the first mitotic cell division [10–15] (Fig. 1).

De novo protein synthesis is required to activate and then sustain zygote gene expression and so represents a critical first step for initiating embryonic development [16]. Interestingly, the form and character of protein expression is influenced by the frequency and duration of Ca^{2+} spikes [17] raising the possibility that CamKII transduces these events towards a programmed induction of translation. Since de novo gene transcription does not occur during oocyte MII arrest, the first phase of protein synthesis occurs by translation of mRNA derived from the maternal genome, retained in the oocyte as stable dormant transcripts through sexual maturity and ovulation. Cytoplasmic polyadenylation binding element protein-1 (CPEB-1) plays a central role in this process by mediating the silencing, storage or activation of maternal transcripts through its interaction with 3′-untranslated mRNA regions. Studies in zebra fish demonstrate that CPEB isoforms mediate a cascade of events from initial activation of the transcriptome in the fertilised oocyte through to the establishment of tissue patterns during embryogenesis [18]. A more detailed analysis of this cascade in fertilised mammalian embryos revealed CPEB1 initiates a positive feedback loop involving up-regulation of RNA binding proteins including DAZL (deleted in azoospermia-like) whose expression is essential for regulating other proteins involved in mitotic spindle assembly and function [19]. CPEB-1 is also a calcium-activated CamKII substrate whose phosphorylation in non-embryonic tissues has been shown to promote its interaction with 3′ untranslated region (UTR) of mRNA, induce its polyadenylation and so increase the efficiency of gene expression [20,21]. Though yet to be demonstrated in 1-cell embryos, this protein seems to show the type of acutely responsive calcium-dependent regulation that could co-ordinate calcium signalling intensity with the expression of distinct maternal mRNAs as the first step in protein synthesis.

Protein synthesis cannot occur, however, without ribosome assembly and the initiation of a translational cap complex and so CEBP-1 function must be partnered with regulators of this process. mTOR is well established as the kind of nutrient-sensitive regulator of cell growth which fits beautifully with Brailsford-Roberson's concept of an "endogenous, nutrient-sensitive autocatakinetic growth regulator". mTOR forms the catalytic core of two distinct multi-protein complexes, mTORC1 (composed of mTOR, mLST8/GβL and regulatory-associated protein of mTOR (raptor)) and mTORC2 (mTOR, mLST8/GβL and rapamycin-insensitive component of mTOR (rictor)). mTORC1 displays acute sensitivity to the mTOR inhibitor, rapamycin and phosphorylates the key proteins in the initiation of ribosome assembly, (S6 kinase 1 (S6K1) and translational initiation, eIF4E binding protein 1 (4EBP1)). mTORC2,