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Movement of mTORC1 observed for the first time in live cells

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What do proteins and wild bears have in common? Just like tagging wild animals aims to allow researchers to observe and track their natural behaviour, molecular researchers use tags to track the minute movements of proteins in cells. Despite the difference in the size of the target, the challenge remains the same: how to tag the object without changing its usual behaviour.

For over ten years, this issue has held back research on understanding the dynamics of a key cellular sensor called mTORC1 (mammalian target of rapamycin complex 1), a multi-protein complex. mTORC1 is responsible for regulating cell growth in response to favourable or unfavourable conditions. In circumstances where the cell is experiencing limited nutrients, mTORC1 is inactive which stimulates the cell's reuse and recycle (autophagy) processes. When mTORC1 detects available nutrients, it switches on the appropriate synthesis pathway in the cell to make full use of them, for example, if amino acids (the building blocks of proteins) abound, mTORC1 will trigger protein synthesis. Previous research has found that inhibiting the mTOR subunit of the complex extends lifespan, indicating that mTOR is likely to be the most important regulator of ageing due to its role in governing cell turnover and growth.

Now, for the first time, researchers at the Babraham Institute have been able to successfully tag a protein in this complex to observe its intracellular movement in real time. The discovery of how the complex behaves modifies current thinking about mTORC1 activation and signalling and provides new tools to dissect the role of mTORC1 in

governing cell growth. The research is published in the journal eLife today.

By engineering a fluorescently-tagged and active form of mTORC1 and using the Institute's live imaging capabilities, the researchers were able to track the time course of what happened to mTORC1 in 'starved' cells following the addition of amino acids, one of the nutrients measured by mTORC1. mTORC1 has different cellular locations depending on whether it is active or inactive. On activation, mTORC1 moves from the cell cytoplasm to attach to the surface of lysosomes, cellular membrane sacks which are responsible for digesting proteins and other cellular components. What was not known was how quickly this happened. The researchers found that the movement of mTORC1 to the lysosome membrane occurred within two minutes of amino acids being added to the cell media and that mTORC1 detaches again after about three to four minutes.

Dr Nicholas Ktistakis, group leader in the Institute's Signalling research programme and senior author on the paper, said: "An active tagged version of mTORC1 provides a significant new tool we can use to observe the real-time dynamics of mTORC1 and further probe the complexities of mTORC1 signalling. Discovering the speed of mTORC1 relocation to lysosomes was really astonishing and when we combine this with data showing the time period of mTOR kinase activity we can see that this requires a rethinking of our existing models and raises new questions."

In addition to labelling mTORC1, the researchers utilised the Institute's biological chemistry expertise to produce a fluorescently labelled amino acid (leucine). This is the first fluorescent reagent capable of activating mTORC1 and visible by microscopy. Using both fluorescently labelled mTORC1 and leucine allowed the real time observation of both amino acid entry to lysosomes and subsequent mTORC1 movement in the cell.

Dr Maria Manifava, senior researcher at the Babraham Institute and joint first author on the paper, said: "By providing a time-dependent activity pattern of mTORC1 in relation to its dynamic localisation, we found that there is a population of mTORC1 in the cell which is no longer on the lysosomes but nevertheless active. To explain this, we propose that the localisation of mTORC1 to the lysosomes somehow modifies it to maintain its activity even when it detaches again." Matthew Smith, a PhD student at the Babraham Institute at the time and joint first author on the paper, continued: "Our next steps are to identify the effect of mTORC1's interaction with the lysosome structure that enable it to maintain its activity after detaching. Knowing this will allow a more complete picture of the steps involved in amino acid sensing by mTORC1."

More information: Maria Manifava et al. Dynamics of mTORC1 activation in response to amino acids, *eLife* (2016). DOI: 10.7554/eLife.19960

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