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ORIGINAL RESEARCH

Direct imaging of S6K1 recruitment and phosphorylation in the mTORC1 pathway in living cells - Evidence review

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1. ABSTRACT

The analyzed study examined how the S6K1 protein, a key executor of cell growth signals, is organized and activated by the mTORC1 complex in living cells. Using fluorescence microscopy and a biosensor constructed by the authors, the research observed that S6K1 is distributed in both the nucleus and the cytoplasm, but is shifted primarily to the cytoplasm when co-expressed with raptor, an organizational component of mTORC1. Under these conditions, the phosphorylation of S6K1—that is, the addition of a chemical group that promotes its activation—was found to be localized mainly in the cytoplasm.The publication contributes to the passage from “The Choice to Live in Health or Illness” by Dr. A. Colonnese and R. Panzironi, which states that S6K1 must be recruited near mTORC1 before phosphorylation. Independent studies confirm that the TOS motif of S6K1 allows for recognition by raptor and that the availability of raptor influences phosphorylation. Structural data further clarify the contact point between raptor and the TOS motif. However, there is an alternative interpretation, based on cellular experiments, which attributes a direct role to eIF4E in the interaction with the TOS motif. Overall, the narrative is confirmed primarily at the molecular and cellular level.

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2. ANALYZED STUDY

The research question was to understand where, inside a living cell, S6K1 is recruited by the mTORC1 complex and where its phosphorylation occurs. mTORC1 (a protein complex that integrates signals from nutrients and growth factors) can activate S6K1, a kinase, which is a protein that modifies other proteins by adding phosphate groups. The authors hypothesized that raptor, a component of mTORC1 that acts as an organizational platform, facilitates the recruitment of S6K1 and its localization in the cytoplasm.The relevance to section 4.7 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi is specific: the book states that, before being phosphorylated, S6K1 must be recruited near mTORC1 through a precise organization of the signaling platform proteins. The analyzed study directly observes, in living cells, the cellular segment of this chain: functional association between S6K1, raptor, and mTORC1, redistribution of S6K1 in the cytoplasm, and predominantly cytoplasmic phosphorylation.The research can therefore document molecular dynamics in a cellular experimental model. It cannot establish that the same sequence of events occurs identically in all human tissues, nor can it demonstrate clinical, metabolic, or therapeutic consequences on its own.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The study is experimental and primarily used HEK293 cells, human embryonic kidney cells cultured in the laboratory. HEK293T cells, suspension HEK293F cells, and SF9 insect cells were also used to produce and purify the biosensor. HEK293 cells were seeded at a density of 1 × 105 or 1.5 × 105 cells per mL and transfected—that is, induced to produce artificially introduced proteins—with plasmids encoding S6K1, raptor, mTOR, Rheb, and other fluorescent variants.Confocal microscopy and FRET-FLIM were used to visualize proteins and interactions. FRET-FLIM (a method that detects whether two fluorescent molecules are close enough to change the duration of their light emission) allows for the estimation of protein-protein interactions in living cells. The authors also performed Western blots, a technique that measures the presence and phosphorylation state of proteins, pull-down experiments to isolate protein complexes, and immunofluorescence on fixed cells.A biosensor called SensOR was constructed, consisting of mCherry-S6K1-EGFP. The sensor exploits the conformational change of S6K1 following phosphorylation: a more folded structure brings the fluorescent markers closer together and shortens the fluorescence lifetime. To modify the mTORC1 signal, cells were deprived of serum and amino acids, then exposed to 1 mM leucine or 500 mM serine plus leucine; finally, 250 nM rapamycin was added. In another experiment, 200 nM rapamycin was used. The results are described as representative of three independent experiments, unless otherwise indicated. For the high-expression SensOR analysis, more than 40 cells were examined across three independent experiments.No inclusion or exclusion criteria, sample size calculation plan, clinical endpoint, or follow-up are reported, as this is a laboratory study on cells and proteins.

4. RESULTS

Under basal conditions, EGFP-tagged S6K1 was present in almost equal amounts in the cytoplasm and nucleus of HEK293 cells: 54% of the fluorescent intensity was cytoplasmic and 46% was nuclear, with a standard deviation of 10%. This finding indicates that the protein is not stably confined to a single cellular compartment in the model studied.When S6K1 was co-expressed with tagged raptor, S6K1 became predominantly cytoplasmic. The increase in the cytoplasmic fraction of S6K1 was proportional to the level of raptor in the cytoplasm. FRET-FLIM microscopy further showed that the fluorescence lifetime of EGFP-S6K1 shifted from 2.6 ± 0.01 nanoseconds when S6K1 was expressed alone to 2.4 ± 0.05 nanoseconds in the presence of raptor, with a reduction down to 2.3 ± 0.03 nanoseconds in the cytoplasm. A shorter lifetime is consistent with greater physical proximity between the two markers and supports direct interaction between S6K1 and raptor in living cells.The F28A mutation in the TOS motif, a short sequence of S6K1 involved in recognition by mTORC1, prevented the cytoplasmic redistribution induced by raptor. Co-expression of PRAS40 or 4E-BP1, proteins that can compete for interaction with raptor, also reduced S6K1 translocation. These results

are consistent with the idea that recruitment depends on the organization of the protein platform and not on the simple random presence of S6K1 in the cytoplasm.Rheb, an upstream regulator of mTORC1, increased S6K1 phosphorylation more than twofold. In the presence of Rheb, treatment with 200 nM rapamycin reduced this phosphorylation by approximately 50%. The authors did not detect a strong direct interaction between S6K1 and Rheb and observed only a slight reduction in the fluorescence lifetime between mTOR and S6K1; the result is compatible with weak or dynamic contact but does not prove a stable direct bond.With the SensOR biosensor, high-expression cells showed an average lifetime of 2.1 ± 0.06 nanoseconds in the cytoplasm and 2.3 ± 0.01 nanoseconds in the nucleus. After serum and amino acid deprivation, the cytoplasmic lifetime was 2.5 ± 0.03 nanoseconds; the addition of amino acids reduced it to 2.3 ± 0.02 nanoseconds, while rapamycin returned it to 2.5 ± 0.04 nanoseconds. In a purified preparation, the addition of ATP reduced the lifetime from 2.6 ± 0.01 to 2.0 ± 0.08 nanoseconds and increased biosensor phosphorylation by 4.7-fold. In the experimental model, these data support the association between the folded conformation, phosphorylation, and cytoplasmic localization of S6K1.

5. INTERPRETATION

In simple terms, the results describe a sequence in which S6K1 does not appear to be activated indiscriminately throughout the cell. Instead, Raptor appears to be a protein that helps position S6K1 near the mTORC1 complex, especially in the cytoplasm, making phosphorylation more likely. The specifically documented point is therefore the cellular recruitment of S6K1 in relation to Raptor and the functional structure of mTORC1.The molecular detail is that the TOS motif of S6K1 is necessary for the redistribution observed with Raptor, and that the F28A mutation reduces this phenomenon. Furthermore, the reduction in fluorescence lifetime in FRET-FLIM is consistent with the proximity between S6K1 and Raptor. The biosensor indicates that S6K1 phosphorylation occurs primarily in the cytoplasm under the conditions used.The main interpretative limitation is that the study uses overexpression of fluorescent proteins in cell lines. This choice enables real-time imaging but may alter the quantities, ratios, and localization of proteins compared to non-manipulated cells. The observed translocation supports a recruitment model, but it does not prove that it is mandatory in every cell type or in all physiological contexts. The publication does not demonstrate the entire chain described in the book, such as the subsequent effects on protein synthesis, growth, or metabolism: it documents the initial step of organization and the approach of S6K1 to the mTORC1 platform.

6. INDEPENDENT SUPPORTING EVIDENCE

Initial independent confirmation comes from a cellular and biochemical study that analyzed the TOS motif of S6K1. The authors showed that raptor binds S6K1 and 4E-BP1 through their respective TOS motifs and that a point mutation in the motif eliminates the raptor-dependent phosphorylation component of S6K1. The result directly confirms the passage in the book stating that recruitment requires a precise organization of platform proteins. The limitation is that the evidence is derived from in vitro experiments and cultured cells, not from measurements in the human organism [1].A second paper provided structural verification. Using crystallography and cryo-electron microscopy, the authors obtained structures of raptor bound to peptides containing the human TOS motif of S6K1, 4E-BP1, and PRAS40. The S6K1 peptide occupied a groove in raptor; mutations in key residues reduced binding. This result does not observe translocation in living cells, but it independently confirms the physical basis of the recognition between raptor and the TOS motif of S6K1 [2].A third study showed that recruitment is also regulated by competition between substrates. In mouse liver and embryonic fibroblasts lacking 4E-BP1 and 4E-BP2, the phosphorylation of p70S6K1 at Thr389 after feeding was increased 22-fold, compared to seven-fold in controls; in fibroblasts, IGF-1-induced phosphorylation was nearly doubled. The increase was associated with greater interaction between S6K1 and raptor. Since 4E-BP1 competes for raptor, the study shows that S6K1 recruitment depends on the dynamic composition of the mTORC1 platform and not exclusively on the total amount of mTOR [3].Taken together, these studies support a convergent chain: the TOS motif allows for recognition, raptor provides a docking site, and the availability of other

substrates can modulate how much S6K1 reaches the active complex. These are independent confirmations of the cellular mechanism described by the publication subject to the Evidence Review, but they remain primarily molecular, cellular, and animal evidence.

7. CONFLICTING EVIDENCE

A study published in 2021 proposes an alternative interpretation of the role of the TOS motif. In HEK293 cells and tumor cell lines, the authors observed that phosphorylated eIF4E, a protein involved in the initiation of messenger RNA translation, can interact with the TOS motif of S6K1. According to this model, the interaction with eIF4E would reduce the autoinhibition of S6K1 and promote its activation; the authors argue that the binding previously attributed directly to raptor may be, at least in part, a consequence of this step [4].This evidence does not deny that raptor is an essential component of mTORC1, nor does it exclude the interactions documented by biochemical and structural studies. However, it suggests that the process may be more complex and dependent on the experimental context, with eIF4E acting as a possible intermediary or additional regulator. The work is based on cell models, mutants, and gene silencing; it therefore requires confirmation from independent groups and does not demonstrate a generalized replacement of raptor's role in human tissues.

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the publication reviewed from the independent evidence used to confirm or define the scope of the book's passage.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Cellular and biophysical	Live HEK293 cells; fluorescent proteins and SensOR biosensor	Raptor promotes the cytoplasmic localization of S6K1; biosensor phosphorylation is primarily cytoplasmic.	direct confirmation	moderate
[1]	Cellular and biochemical	Cultured cells and in vitro assays	Raptor binds S6K1 through the TOS motif; mutation of the motif reduces raptor-dependent phosphorylation.	direct confirmation	moderate
[2]	Structural and biochemical	Purified protein complexes; human TOS peptides	The structure of raptor directly recognizes the S6K1 TOS motif.	direct confirmation	moderate
[3]	Animal and cellular	Mouse liver and mouse embryonic fibroblasts	The absence of 4E-BP1/2 increases S6K1-raptor interaction and accelerates S6K1 phosphorylation.	indirect confirmation	moderate

[4]	Cellular and molecular	HEK293 cells and tumor cell lines	Proposes that phosphorylated eIF4E interacts with the S6K1 TOS motif and contributes to its activation.	discordant evidence	preliminary

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The analyzed study specifically argues that S6K1 is not phosphorylated as an isolated protein, but is organized near mTORC1 through platform components, especially raptor. Independent evidence confirms that the TOS motif is important for S6K1 recognition and that the availability of raptor and competing substrates influences phosphorylation. The conclusion is well-supported at a mechanistic level, but requires direct validation in human tissues and under physiological conditions not based on protein overexpression. The alternative hypothesis involving eIF4E also suggests that recruitment may include additional regulators.

Aggiornato al 17/11/2026. Questo contenuto riflette una sintesi divulgativa delle evidenze scientifiche al momento della pubblicazione e non sostituisce il parere medico.

10. TRANSPARENCY AND SCIENTIFIC RESPONSIBILITY

Artificial Intelligence was used solely as a tool for editorial and documentary support. All content, interpretations, bibliographic citations and conclusions have been verified, reviewed and approved by the author, who assumes full scientific responsibility for them.

The manuscript was also subjected to independent peer review prior to publication.

To ensure the greatest transparency, the complete history of the article, including the dates of submission, revision, acceptance and publication, as well as information on the peer review process, is available on the dedicated Article History page.

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