CDB SEMINAR

Speaker: Kazuyoshi Yonezawa

<Professor, Biosignal Research Center, Kobe University>

Title: "Cellular Functions and Molecular Mechanisms of Signaling Pathways Mediated by Target of Rapamycin"

Date: Monday, June 23

Time: 16:00 P.M.~18:00 P.M.

Place: 7th floor Conference Room, CDB

Summary

The mammalian target of rapamycin (mTOR) is a protein kinase that controls multiple cellular functions in response to amino acids and growth factors, in part by regulating the phosphorylation of p70 S6 kinase (p70S6k) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1). The mechanism by which mTOR regulates p70S6k and 4E-BP1 in vivo remain incompletely understood. We found that raptor (regulatory associated protein of mTOR) is a 150 kDa mTOR binding protein that also binds 4E-BP1 and p70S6k. The binding of raptor to mTOR is necessary for the mTOR-catalyzed phosphorylation of 4E-BP1 and p70S6k in vitro. Rapamycin or amino acid withdrawal increases, whereas insulin strongly inhibits, the recovery of 4E-BP1 and raptor on 7-methyl-GTP Sepharose. Partial inhibition of raptor expression by RNA interference (RNAi) reduces mTOR-catalyzed 4E-BP1 phosphorylation. In addition, we demonstrates that raptor binds to p70S6k and 4E-BP1 through their respective TOS (conserved TOR signaling) motifs, a short conserved segment previously shown to be required for amino acid- and mTOR-dependent regulation of these mTOR substrates in vivo. A point mutation within the TOS motif of p70S6k or 4E-BP1 known to abolish both amino acid- and mTOR-regulation, selectively abolishes their binding to raptor. This mutation of the TOS motif also eliminates all in vitro mTOR-catalyzed 4E-BP1 phosphorylation and abolishes the raptor-dependent component of mTOR-catalyzed p70S6k phosphorylation in vitro. Raptor does not alter mTOR's intrinsic catalytic activity, but appears to serve as an mTOR scaffold protein whose binding to the TOS motif of mTOR substrates is necessary for effective mTOR-catalyzed phosphorylation in vivo and perhaps for conferring their sensitivity to rapamycin and amino acid sufficiency.

Host **Shigeo Hayashi** **Morphogenetic Signaling ,CDB**

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