· which are features of both pi3k

· The , and FAT domain structure. The is a human mTOR with a truncated N-terminal that is bound mLST8. The kinase activity of is comparable to that of mTORC1, however the complex is more active at higher substrate concentrations, which is opposite to the mTORC1 (Yang et al.

, 2013). The has a compact shape with a FAT domain, which consist of an alpha-alpha helical repeats that forms a C-shaped solenoid which wraps half way around the kinase domain and clamps into it. The FATC domain is integral to the kinase domain structure. However, the mLST8 and the FRB domain extend beyond the kinase domain, thus are found on the opposite sides of the catalytic cleft (Yang et al., 2013)(Fig. 5a). The mTOR kinase domain consists of the two-lobe structure, the N-terminal lobe (N-lobe) and the C-terminal lobe (C-lobe), which are features of both PI3K and PK families. Between the N-lobe and the C-lobe there is cleft that binds to ATP (Baretic et al.

, 2014). The FRB domain, with the residue insertion, is inserted within the kinase N-lobe as well as a 40-residue insertion in the C-lobe that forms the binding site for mLST8 (Fig. 5a). The mTOR kinase domain structure starts before the FRB domain with the long k1 helix integrated to the structure of the N-lobe.

The FRB insertion occurs right after the k1 helix followed by a short beta-strands and two short helices that pack the FRB (Yang et al., 2013). The C-lobe contains the catalytic cleft with four structural insertions (LBE, k9b, and FATC; Fig. 3b).

These will form a center of interaction ate the activation loop that is important for the regulation of PK. The activation loop, which is well ordered on mTOR structure, is believed to have a comparable role in PI3Ks among which it is ordered in only the class III PIK3C3 structures (Okkenhaug et al., 2013). The FATC makes interactions with the activation loop suggesting that it may have a role in stabilizing the activation loop structure and the LBE (Fig. 3b). mLST8 consists of a seven Beta-propeller that extends the WD40 repeats and binds to both helices and the intervening loop of the LBE.

mLST8 is thought to be a requisite activating subunit of mTOR complex where its surface may directly stabilize the LBE structure and indirectly influence the organization of the active site through the LBE/FATC/activation-loop spine of interaction (Yang et al., 2013). The FAT domain contain 28 alpha helices where alpha1 –alpha22 belong to the TRP repeat family and form three discontinuous domains (TRD1, TRD2, and TRD3). The contacts of TRD1 and HDR to the KD are important for the function and structure of mTOR (Fig. 5b).

The TRD1 and HDR segments correspond to the FAT segments, which are conserved within the PIKK family members. The FAT domain clamping onto the KD is a common feature of this family (Baretic et al., 2014).

· Comparison of the mTOR catalytic center to PI3Ks. In the PI3Ks and other Pks, the N-lobe of the mTOR kinase domain is smaller. It is composed of five Beta-sheets associate with few alpha-helices while the C-lobe mostly contain alpha-helices. The active site and the ATP-binding region of the mTOR are located between the N- lobe and the C-lobe. In mTOR, the kinase

domain N-lobe is structurally more similar to PI3Ks than Pks. In PI3Ks and mTOR the N-lobe packs against the HEAT repeats of the helical FAT domain (Baretic et al.

, 2014). Among kinase families, there is a conserved element of the active site known as the P-loop that is found close to the N-terminus of the conserved N-lobe. Residues from this loop interact with ATP via the gamma phosphate groups and these interactions are conserved among mTOR and PI3Ks. In mTOR, the P-loop has a conserved serine, which coordinates the beta-phosphate of ATP that is also seen in PI3Ks (Fig. 6). Moreover, mTOR also has another conserved residue, lysine that is covalently modified by wortmannin in mTOR and the PI3Ks (Baretic et al., 2014).

The amphipathic helix serves as basal element of the active site in the protein kinases. In the mTOR, the hydrophilic side of the is exposed toward the ATP-binding site residues and the FAT domain while the hydrophobic side packs with helices 6/8/9 (Fig. 6). In the PI3Ks, the helix makes equivalent interactions with the ATP-binding site, 8/9 and the helical domain Gln711-Gln400 (Yang et al., 2013). The C-lobe of the mTOR contains the majority of the active site. The mTOR activation loop has two conserved motifs at the N-lobe and the C-lobe, HIDFG with the highly conserved DFG motif is present at the N-terminal end of the activation loop of both PI3Ks. From the structure of the PI3Ks (Fig.

6), the catalytic and the activation loops are locked in different conformations. In the recent crystal structure of mTOR complex bound to ADP-Mg-F (Fig. 6), the conserved residues of both DRHN and the DFG motifs

are positions toward the active site, which indicates that the mTOR is an intrinsically active enzyme (Yang et al., 2013). For the PI3K family, no equivalent change in conformation has been observed and they are not regulated by activation loop phosphorylation. The FATC has and a conserved among the mTOR orthologous at the end of the C-lobe (Fig. 6). The FATC is stabilized through interactions with the active loop on one side and a hydrophobic interface with the LBE on the other side.

The loop uses the C-terminal motif to clamp the hinge of FATC onto the LBE and where the bond to mLST8 (Yang et al., 2013). In this way the activation loop forms a cage structure around the FATC hinge, stacking its phenylalanine perpendicular to the tyrosine, which helps to position hydrophobic patch of toward the active site. The structural organization of the mTOR helices , , and resembles the C-terminal regulatory arch composed of the equivalent helices in PI3Ks (Fig. 6).

In PI3Ks, helix sits on the surface of the C-lobe where it reflect an active to inactive sate transition while in the mTOR it is hidden behind the LBE and it does not seem to be involved in an open-to closed transition (Baretic et al., 2014). Moreover, the mTOR structure and in vitro kinase activity suggest that the conformation of the kinase domain with the folded inside the C-lobe is inherently open and active in the absence of the regulatory subunits such as he RAPTOR and RICTOR/mSIN1 (Saxton et al., 2017). Furthermore, the helices , and together with C-terminal regulatory arch in mTOR contains a unique fourth helix b that is conserved among mTOR orthologues and not present in PI3Ks (Yang et al., 2013).

The b helix packs against the activation loop, where it caps the catalytic site and overlaps with the negative regulatory domain (RD is a region of residues between 2430-2450). A deletion of the RD causes an increase of the mTOR kinase activity, but that is believed is due to b since its removal decreases mTOR kinase activity (Yang et al., 2013). One theory suggest that the shortening the linker between helices b and causes tighter packing of the two helices with the activation loop which limits accessibility to the active site. As in P (Fig. 6), a number of the mTOR activating mutations are found along the regulatory arch, thus it is believed that Ras homolog enriched in the brain (RHEB) activates mTOR by interacting with the kinase domain active site, mLST8, and RAPTOR (Saxton et al., 2017). · The ATP-binding pocket and the catalytic loop.

The , a highly conserved sequence motif, is found in the active site of the mTOR kinase domain (Fig. 7a). The lysine residue makes interaction with the alpha and /or the beta of the phosphate group of ATP.

The residues following the lysine () forms a connecting loop between and marked by RQD residues. The loop residues contribute to the ATP-binding pocket (Sauer et al., 2013). The Glycine 2188 residue allows for the close packing of the first helix of kinase (). Histidine 2189 donates hydrogen bonds to a conserved residue in the ATP-loop, glutamine 2167. Aspartic acid 2191 contributes to the overall orientation of the GHEDL loop as well as provides polar contact for the side chain arginine 2193 that is located at the N-terminal end of (Sauer et al.

, 2013). Leucine 2192 provides a binding site for the strand, the residues of which form the hydrophobic pocket for the adenine ring. helix is important for the organization of the kinase domain. It is located at the beginning of the C-lobe (Fig. 6).

The first three residues, , are centered at the kinase domain and tend to interact with residues involved in ATP-binding site. The arginine 2193 is involved in the orientation of the Aspartic acid 2191in the GHEDL loop (Sauer et al., 2013). Moreover the aspartic acid 2195 contacts the backbone nitrogen of the activation loop phenylalanine 2358, which is involved in the stacking platform for Recently, this motif has been shown to play an important role as a binding surface for the helix (Sauer et al., 2013). The catalytic loop has a signature motif made of (Sauer et al., 2013).

The homology model found that the triplet is always followed by Proline 2341, which defines a unique feature of the TOR family (Fig. 8 blue). Pro2341 forms a hydrophobic core at the C-terminal end of the catalytic loop. It is centered on Trp2549, the last residue of mTOR. The mTOR have three important loops that are vital for its catalytic activity: the activation loop, the catalytic loop, and the P-loop. The activation loop is part of the polypeptide-binding site, which carried the DFG motif with the Asp interacting with the cofactor at the active site. The catalytic loop carries three important residues, DHN, that area involved in the catalytic reaction (Sauer et al., 2013).

Aspartic acid is involved in the orientation of the substrate as well as the polarization of the hydroxyl group. The histidine is involved in stabilizing the

gamma phosphate transition state and the asparagine stabilizes the second metal ligand. · FRB role in mTOR kinase domain. Acute rapamycin treatments inhibit the catalytic activity and signaling capacity of mTORC1 while it fails to inhibit mTORC2. The rapamycin-binding site maps to the FRB surface closest to the active site, suggesting that the rapamycin-binding site interacts with substrates to facilitate their entry to the active site. Also, S6K1 and 4EBP1 contain a TOR signaling (TOS) motif that mediates essential interaction with the scaffolding protein raptor to facilitate the recruitment of substrates to the mTOR kinase (Yang et al., 2013).

In order to map the region of S6K1 that is involved in FRB interactions, that region was deleted which led to the reducing of Thr 389 phosphorylation. The data obtained from that experiment indicated that the FRB provides a secondary substrate-recruitment site near the entrance of the catalytic cleft and they presumed that, although TOS motif, is the primary means of substrate recruitment, the secondary site may also facilitates substrate entry into the restricted active site as well as it can provide more specificity for the substrates (Yang et al., 2013). Furthermore, Rapamycin inhibits mTOR activity in a substrate and phosphorylation-site dependent manner. The tertiary complex, which consists of the rapamycin, FKBP12, and other conserved residues of the mTOR FRB domain forms just in front of the catalytic cleft, which constrict access to the active site (Baretic et al.

, 2014). It is also suggested that the rapamycin binds to a conserved secondary substrate site on the FRB meaning that rapamycin is actually a competitive inhibitor for the protein substrate.