UNIVERSITÀ DEGLI STUDI DI MILANO

CONCORSO PUBBLICO, PER TITOLI ED ESAMI, A N. 1 POSTO DI CATEGORIA D - AREA TECNICA,TECNICO-SCIENTIFICA ED ELABORAZIONE DATI, TECNICO PER LA PIATTAFORMA DI TECNOLOGIE AVANZATE, CON RAPPORTO DI LAVORO SUBORDINATO A TEMPO INDETERMINATO PRESSO IL DIPARTIMENTO DI BIOSCIENZE, DA RISERVARE, PRIORITARIAMENTE, ALLE CATEGORIE DI CUI AL DECRETO LEGISLATIVO N. 66/2010 – CODICE 20768

La Commissione Giudicatrice del concorso, nominata con determina n. 6155 del 29/05/2020, composta da:

Prof. Minucci Saverio - Presidente

Prof. Vaccari Thomas - Componente

Dott.ssa Ascagni Miriam - Componente

Sig. Montana Giuseppe - Segretario

comunica i quesiti relativi alla prova orale:

GRUPPO DI QUESITI NR. 1

1. Il candidato descriva le potenzialità e i limiti di un programma Open Source per l'analisi di immagini
2. Il candidato spieghi quali accortezze metterebbe in atto per prevenire eventuali danni agli obbiettivi di un microscopio inserito nel contesto di una facility di microscopia
3. Il candidato spieghi il funzionamento della strumentazione necessaria per un'analisi di microscopia a fluorescenza su molti campioni cellulari in parallelo
4. Brano in inglese: Il candidato legga e traduca il seguente Abstract:

   The mechanistic target of rapamycin complex 1 (mTORC1) is a key metabolic hub that controls the cellular response to environmental cues by exerting its kinase activity on multiple substrates[1,2,3]. However, whether mTORC1 responds to diverse stimuli by differentially phosphorylating specific substrates is poorly understood. Here we show that transcription factor EB (TFEB), a master regulator of lysosomal biogenesis and autophagy[4,5], is phosphorylated by mTORC1 via a substrate-specific mechanism that is mediated by Rag GTPases. Owing to this mechanism, the phosphorylation of TFEB—unlike other substrates of mTORC1, such as S6K and 4E-BP1— is strictly dependent on the amino-acid-mediated activation of RagC and RagD GTPases, but is insensitive to RHEB activity induced by growth factors. This mechanism has a crucial role in Birt–Hogg–Dubé syndrome, a disorder that is caused by mutations in the RagC and RagD activator folliculin (*FLCN*) and is characterized by benign skin tumours, lung and kidney cysts and renal cell carcinoma[6,7]. We found that constitutive activation of TFEB is the main driver of the kidney abnormalities and mTORC1 hyperactivity in a mouse model of Birt–Hogg–Dubé syndrome. Accordingly, depletion of TFEB in kidneys of these mice fully rescued the disease phenotype and associated lethality, and normalized mTORC1 activity. Our findings identify a mechanism that enables differential phosphorylation of mTORC1 substrates, the dysregulation of which leads to kidney cysts and cancer.

GRUPPO DI QUESITI NR. 2

1. Il candidato elenchi i più comuni formati utilizzati per il salvataggio delle immagini e spieghi quale ritiene sia il più adatto e per quali motivi
2. Il candidato elenchi e illustri brevemente le principali aberrazioni delle lenti in microscopia ottica
3. Il candidato spieghi quali criteri e ottimizzazioni perseguirebbe per adattare un esperimento di microscopia ottica a fluorescenza a sistemi high throughput
4. Brano in inglese: Il candidato legga e traduca il seguente Abstract:

   GABA (γ-aminobutyric acid) stimulation of the metabotropic $GABA_B$ receptor results in prolonged inhibition of neurotransmission that is central to brain physiology[1]. $GABA_B$ belongs to the Family C of G protein-coupled receptors (GPCRs), which operate as dimers to relay synaptic neurotransmitter signals into a cellular response through the binding and activation of

heterotrimeric G proteins[2,3]. $GABA_B$, however, is unique in its function as an obligate heterodimer in which agonist binding and G protein activation take place on distinct subunits[4,5]. Here we show structures of heterodimeric and homodimeric full-length $GABA_B$ receptors. Complemented by cellular signaling assays and atomistic simulations, the structures reveal an essential role for the $GABA_B$ extracellular loop 2 (ECL2) in relaying structural transitions by ordering the linker connecting the extracellular ligand-binding domain to the transmembrane region. Furthermore, the ECL2 of both $GABA_B$ subunits caps and interacts with the hydrophilic head of a phospholipid occupying the extracellular half of the transmembrane domain, thereby providing a potentially crucial link between ligand binding and the receptor core that engages G protein. These results provide a starting framework to decipher mechanistic modes of signal transduction mediated by $GABA_B$ dimers and have important implications for rational drug design targeting these receptors.

GRUPPO DI QUESITI NR. 3

1. La DEPHT di un'immagine: il candidato spieghi le differenze tra un'immagine 8bit e un'immagine 12 bit
2. obbiettivi a secco, ad immersione ad olio e ad immersione ad acqua: il candidato spieghi le differenze tra questi tipi di obbiettivi ed indichi quali di questi preferirebbe utilizzare, motivando la sua scelta, nel caso di:
   - Studi in vivo su cellule overnight
   - Studi su supporto in plastica
   - Studi su campioni con marcatura poco espressa
3. Il candidato spieghi come organizzare su una piastra multi pozzetto campioni e controlli in un esperimento di high content screening
4. Brano in inglese: Il candidato legga e traduca il seguente Abstract:

   Nuclear processes, such as V(D)J recombination, are orchestrated by the three-dimensional organization of chromosomes at multiple levels, including compartments1 and topologically associated domains (TADs)2,3 consisting of chromatin loops4. TADs are formed by chromatin-loop extrusion5,6,7, which depends on the loop-extrusion function of the ring-shaped cohesin complex8,9,10,11,12. Conversely, the cohesin-release factor Wapl13,14 restricts loop extension10,15. The generation of a diverse antibody repertoire, providing humoral immunity to pathogens, requires the participation of all *V* genes in V(D)J recombination16, which depends on contraction of the 2.8-Mb-long immunoglobulin heavy chain (*Igh*) locus by Pax517,18. However, how Pax5 controls *Igh* contraction in pro-B cells remains unknown. Here we demonstrate that locus contraction is caused by loop extrusion across the entire *Igh* locus. Notably, the expression of Wapl is repressed by Pax5 specifically in pro-B and pre-B cells, facilitating extended loop extrusion by increasing the residence time of cohesin on chromatin. Pax5 mediates the transcriptional repression of *Wapl* through a single Pax5-binding site by recruiting the polycomb repressive complex 2 to induce bivalent chromatin at the *Wapl* promoter. Reduced Wapl expression causes global alterations in the chromosome architecture, indicating that the potential to recombine all *V* genes entails structural changes of the entire genome in pro-B cells.

GRUPPO DI QUESITI NR. 4

1. Il candidato spieghi in cosa consiste il processo di deconvoluzione delle immagini
2. Il candidato spieghi che tipo di considerazioni farebbe per gestire ed organizzare un alto flusso di utenti con esperimenti diversi su un microscopio condiviso
3. Il candidato spieghi come impostare un esperimento di valutazione in parallelo dell'efficacia di composti farmacologici su una popolazione di cellule
4. Brano in inglese: Il candidato legga e traduca il seguente Abstract:

   In metazoans, the secreted proteome participates in intercellular signalling and innate immunity, and builds the extracellular matrix scaffold around cells. Compared with the relatively constant intracellular environment, conditions for proteins in the extracellular space are harsher, and low concentrations of ATP prevent the activity of intracellular components of the protein quality-control machinery. Until now, only a few bona fide extracellular chaperones and proteases have been shown to limit the aggregation of extracellular proteins1,2,3,4,5. Here we performed a systematic analysis of the extracellular proteostasis network in *Caenorhabditis*

elegans with an RNA interference screen that targets genes that encode the secreted proteome. We discovered 57 regulators of extracellular protein aggregation, including several proteins related to innate immunity. Because intracellular proteostasis is upregulated in response to pathogens[6,7,8,9], we investigated whether pathogens also stimulate extracellular proteostasis. Using a pore-forming toxin to mimic a pathogenic attack, we found that *C. elegans* responded by increasing the expression of components of extracellular proteostasis and by limiting aggregation of extracellular proteins. The activation of extracellular proteostasis was dependent on stress-activated MAP kinase signalling. Notably, the overexpression of components of extracellular proteostasis delayed ageing and rendered worms resistant to intoxication. We propose that enhanced extracellular proteostasis contributes to systemic host defence by maintaining a functional secreted proteome and avoiding proteotoxicity.

Milano, 13.07.2020

La Commissione

Prof. Minucci Saverio - Presidente ……

Prof. Vaccari Thomas - Componente ……

Dott.ssa Ascagni Miriam - Componente ……

Sig. Montana Giuseppe - Segretario ……