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**SELEZIONE N. 2020S4, PER ESAMI, PER L'ASSUNZIONE A TEMPO DETERMINATO DI N. 1 UNITÀ DI CATEGORIA C, POSIZIONE ECONOMICA 1, AREA TECNICA, TECNICO – SCIENTIFICA ED ELABORAZIONE DATI, CON CONTRATTO DI LAVORO SUBORDINATO E CON ORARIO DI LAVORO A TEMPO PIENO, PRESSO L'UNIVERSITÀ DEGLI STUDI DI PADOVA.**

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# Colloquio

## Prova 1

Argomenti oggetto di valutazione::

- Reazione polimerasica a catena (PCR): descrizione della tecnica e applicazioni in campo biomedico
- Tecniche per determinare la struttura di una proteina
- Tecniche di isolamento e purificazione di organelli cellulari

Verifica delle conoscenze:

- *Programmi di videoscrittura: caratteristiche, formati ed esempi*

# jYCaMP: an optimized calcium indicator for two-photon imaging at fiber laser wavelengths

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**Femtosecond lasers at fixed wavelengths above 1,000 nm are powerful, stable and inexpensive, making them promising sources for two-photon microscopy. Biosensors optimized for these wavelengths are needed for both next-generation microscopes and affordable turn-key systems. Here we report jYCaMP1, a yellow variant of the calcium indicator jGCaMP7 that outperforms its parent in mice and flies at excitation wavelengths above 1,000 nm and enables improved two-color calcium imaging with red fluorescent protein-based indicators.**

Two-photon (2P) microscopy has become a leading method for in vivo imaging owing to its optical sectioning capabilities and the increased depth-penetration of near-infrared light in scattering tissue[1]. However, the light sources commonly used for 2P imaging—tuneable titanium-sapphire lasers and parametric oscillators—are costly, require frequent expert maintenance and lack the output power needed for operating several microscopes simultaneously or for high-speed imaging methods that use extended focal patterns[2–5].

Promising alternatives to these traditional light sources, such as high-power industrial ytterbium-doped fiber lasers (YbFLs) and modelocked semiconductor lasers, have shown feasibility for in vivo imaging[2,6] and are becoming widely available at costs orders of magnitude lower and/or power outputs orders of magnitude higher than conventional tunable lasers (Supplementary Fig. 1). Since the bulk of a 2P microscope's cost is the laser, these sources promise to make 2P imaging accessible to many more users. However, commercially available lasers of this kind are largely limited to a fixed residue to form a π-stacking interaction with the phenolic ring of the GYG-chromophore (compared to the green TYG-chromophore) resulting in a shift toward longer wavelengths[7]. To redshift jGCaMP7, we first introduced mutations that convert GFP into mVenus[8] ('Venus-GCaMP'; jGCaMP7s+M65T, V115Y, K118V, F203L, T222G, V225L, S229A, I250A). Unfortunately, Venus-GCaMP did not exhibit the anticipated spectral shift, retaining excitation and emission spectra similar to its parent GCaMP (Supplementary Fig. 2). To find a truly yellow-fluorescent GCaMP variant, we randomly mutated Venus-GCaMP, and used fluorescence emission ratiometry to screen for spectral shift in bacterial colonies. We found a single amino acid mutation S117P (205 in GFP), close to T115, that produced a pronounced redshift. The resulting variant maintained sensor properties similar to those of the parent GCaMP while exhibiting 19 and 36 nm spectral shift in its one-photon and 2P excitation spectrum, respectively (Fig. 1b and Supplementary Table 1). Similarly, jGCaMP7 variants containing yellow fluorescent protein, YPET and citrine mutations failed to produce yellow emission spectra but were rescued by additional introduction of the S117P mutation (data not shown). In GCaMP, the closed barrel structure of circularly permuted GFP is opened within β-strand 7 to accommodate the calcium-sensing domains. Residues 115 and 117 lie on β-strand 10 of GFP, structurally adjacent to β-strand 7, and their position might be affected by the GCaMP permutation in a way that prevents the crucial π-stacking interaction (Fig. 1a). The substitution S117P might then reorient position 115 to rescue the π-stacking and yield the observed yellow fluorescence.

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## Colloquio

## Prova 2

Argomenti oggetto di valutazione::

- PCR quantitativa: marcatori fluorescenti, analisi dati e applicazioni in campo biomedico
- Descrizione di vettori plasmidici e tecniche di clonaggio per l'espressione di proteine ricombinanti.
- Descrizione delle principali vie di trasduzione del segnale

Verifica delle conoscenze

- *Fogli elettronici: caratteristiche, formati ed esempi*

Article

# Loss- or Gain-of-Function Mutations in *ACOX1* Cause Axonal Loss via Different Mechanisms

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## SUMMARY

*ACOX1* (acyl-CoA oxidase 1) encodes the first and rate-limiting enzyme of the very-long-chain fatty acid (VLCFA) β-oxidation pathway in peroxisomes and leads to $H_2O_2$ production. Unexpectedly, *Drosophila* (*d*) *ACOX1* is mostly expressed and required in glia, and loss of *ACOX1* leads to developmental delay, pupal death, reduced lifespan, impaired synaptic transmission, and glial and axonal loss. Patients who carry a previously unidentified, *de novo*, dominant variant in *ACOX1* (p.N237S) also exhibit glial loss. However, this mutation causes increased levels of ACOX1 protein and function resulting in elevated levels of reactive oxygen species in glia in flies and murine Schwann cells. *ACOX1* (p.N237S) patients exhibit a severe loss of Schwann cells and neurons. However, treatment of flies and primary Schwann cells with an antioxidant suppressed the p.N237S-induced neurodegeneration. In summary, both loss and gain of *ACOX1* lead to glial and neuronal loss, but different mechanisms are at play and require different treatments.

## INTRODUCTION

Lipids are critical for neuronal development, synaptic plasticity, and function (Adibhatla and Hatcher, 2008; Tsui-Pierchala et al., 2002). Abnormal lipid metabolism contributes to the pathogenesis of several neurodegenerative disorders, including Alzheimer's disease (Liu et al., 2017; Liu et al., 2015), Parkinson's disease (Lin et al., 2018; Lin et al., 2019), and various diseases associated with glial dysfunction (Chrast et al., 2011; Toshniwal and Zarling, 1992). Mitochondria and peroxisomes have been implicated in some of these neurodegenerative diseases, but the molecular events that underlie the demise of neurons vary widely (Vishwanath, 2016). Some neurodegenerative diseases have been associated with defects in the degradation of fatty acids by β-oxidation, and these disorders share some common features (Eaton et al., 1996; Poirier et al., 2006; Wanders et al., 2010). In yeast

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## Colloquio

## Prova 3

Argomenti oggetto di valutazione::

- Elettroforesi di proteine e acidi nucleici: principi e applicazioni
- Tecniche per lo studio di interazioni proteina-proteina
- Struttura, funzioni e ruolo fisiopatologico dei mitocondri

Verifica delle conoscenze

- *Presentazioni multimediali: caratteristiche, formati ed esempi*

# Molecular mechanisms and cellular functions of cGAS–STING signalling

*Karl-Peter Hopfner*[1,2] ✉ *and Veit Hornung*[1,2] ✉

Abstract | The cGAS–STING signalling axis, comprising the synthase for the second messenger cyclic GMP–AMP (cGAS) and the cyclic GMP–AMP receptor stimulator of interferon genes (STING), detects pathogenic DNA to trigger an innate immune reaction involving a strong type I interferon response against microbial infections. Notably however, besides sensing microbial DNA, the DNA sensor cGAS can also be activated by endogenous DNA, including extranuclear chromatin resulting from genotoxic stress and DNA released from mitochondria, placing cGAS–STING as an important axis in autoimmunity, sterile inflammatory responses and cellular senescence. Initial models assumed that co-localization of cGAS and DNA in the cytosol defines the specificity of the pathway for non-self, but recent work revealed that cGAS is also present in the nucleus and at the plasma membrane, and such subcellular compartmentalization was linked to signalling specificity of cGAS. Further confounding the simple view of cGAS–STING signalling as a response mechanism to infectious agents, both cGAS and STING were shown to have additional functions, independent of interferon response. These involve non-catalytic roles of cGAS in regulating DNA repair and signalling via STING to NF-κB and MAPKs as well as STING-mediated induction of autophagy and lysosome-dependent cell death. We have also learnt that cGAS dimers can multimerize and undergo liquid–liquid phase separation to form biomolecular condensates that could importantly regulate cGAS activation. Here, we review the molecular mechanisms and cellular functions underlying cGAS–STING activation and signalling, particularly highlighting the newly emerging diversity of this signalling pathway and discussing how the specificity towards normal, damage-induced and infection-associated DNA could be achieved.

**Innate immune system**
A heterogeneous system of molecules, signal transducers and cells that has evolved to detect invading microbes, and elicits a first line of antimicrobial defence and activates the adaptive immune system.

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Cell intrinsic recognition and defence systems against foreign genetic material encompass an ancient and fundamental feature of living systems. A first line of defence in mammals is orchestrated by the innate immune system. Germline-encoded pattern recognition receptors (PRRs) detect various pathogen and damage-associated molecular patterns (PAMPs and DAMPs). Their activation elicits signalling cascades that lead to the initiation of cell autonomous defence mechanisms, as well as the production of soluble mediators, such as type I interferons and pro-inflammatory cytokines (BOX 1). Type I interferons play a central role in impeding viral propagation, hence their production is typically governed by PRRs that have evolved to sense viral infection. By inducing the expression of interferon-stimulated genes, type I interferons boost cell autonomous defence mechanisms in an autocrine manner, and furthermore can spread antiviral immunity and activate the adaptive immune system.

Cytosolic DNA is a potent activator of a type I interferon response[1,2] (FIG. 1). Under normal conditions, DNA is confined to the nucleus and mitochondria, and is rapidly degraded by nucleases in the cytosol and endolysosomal compartments. Following infections, for example, increased amounts of intracellular DNA are detected in a pathway that involves cyclic GMP–AMP synthase (cGAS; also known as MB21D1)[3,4], a member of the nucleotidyl transferase (NTase) enzyme family[5] that functions upstream of stimulator of interferon genes (STING). cGAS normally resides as inactive protein in the cell. Upon binding to DNA, cGAS undergoes a conformational change to an active state and produces the second messenger cyclic GMP–AMP (cGAMP) from ATP and GTP[6–8], which is subsequently detected by the cyclic dinucleotide sensor STING[9,10], an ~40-kDa dimeric transmembrane protein at the endoplasmic reticulum (ER)[11]. Binding of cGAMP activates STING, which then translocates to the Golgi and activates TANK-binding kinase 1 (TBK1). TBK1 then phosphorylates itself, STING and, subsequently, the interferon regulatory factor 3 (IRF3) transcription factor.