

# Uncovering the Cellular Target of Agelastatin A

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**McClary et al. (2017) identify the eukaryotic ribosome as a cellular target of agelastatin A, resolving the long-standing mystery surrounding the cytotoxic natural product's mechanism of action. Structural and modeling studies further pinpointed the molecule's binding site to the ribosome peptidyl transferase center, revealing key molecular interactions that drive binding.**

Through natural selection, small-molecule natural products occupy a biologically unique chemical space. For that reason, many such molecules have served pivotal roles as probes for the elucidation of cellular processes and for the discovery of new medicines, and other natural products hold similar promise. However, for this promise to be realized, connecting the phenotypic effects of a natural product to its cellular, mechanistic target is most often essential. Like a detective story, target identification begins with a compilation of potential suspects, and comparison to more well-understood culprits through systems and “top-down” approaches often leads to critical clues (Schenone et al., 2013). In this issue of *Cell Chemical Biology*, McClary et al. shed light on the target and mechanism of the molecule agelastatin A, a cytotoxic natural product that has long intrigued both the synthetic chemistry and chemical biology communities (McClary et al., 2017).

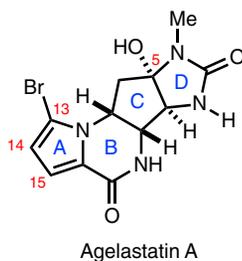
Agelastatin A, first isolated from the sea sponge *Agelas dendromorpha*, is characterized by its halogenated pyrrole-containing tetracyclic structure (Figure 1) (D'Ambrosio et al., 1993). The marine alkaloid exhibits cytotoxic activity against various cancer cell lines, which has been attributed to potential effects in numerous different pathways (Dong, 2010). Although revealing the potential of the agelestatins as anti-cancer agents, these reports have raised important questions as to whether these pleiotropic effects are mechanistically related and what the exact cellular target(s) of agelastatin may be. Together with its complex chemical architecture, these questions have motivated several total syntheses of agelastatin A and its derivatives for further biological studies. Yet despite

these strong interests from both the synthetic and biological communities, the underlying cellular target of the molecule has remained elusive until now. Here, McClary et al. demonstrate that agelastatin A blocks protein synthesis by inhibition of ribosome elongation. Furthermore, they pinpoint and characterize the natural product's binding site in the peptidyl transferase center (PTC) of the ribosome, detailing the molecular interactions between the small molecule and the ribosome through a medley of structural approaches.

Because DNA, RNA, and protein synthesis are essential for cell proliferation, McClary et al. began their studies by profiling the global effects of the agelastatins on those processes, a type of “top-down” approach that has been successfully employed by the team before (Titov et al., 2011). Through this approach, agelastatin A was found to inhibit DNA and protein synthesis in a dose-dependent manner without affecting RNA synthesis. A remarkable similarity between this activity profile of agelastatin A and that of cycloheximide, a well-known inhibitor of ribosome elongation, prompted the authors to further investigate whether the inhibitory effects of agelastatin A on protein synthesis may be direct (Schneider-Poetsch et al., 2010). Through a series of in vitro and cell-based assays, they demonstrate that agelastatin A blocks protein synthesis by directly inhibiting ribosome elongation. Moreover, by taking advantage of synthetic agelastatin analogs, a strong positive correlation between inhibition of protein synthesis and cell growth was observed, supporting the notion that the anti-proliferative activity of agelastatin A is directly linked to inhibition of protein synthesis.

Next, through an impressive battery of structural studies, McClary et al. then identified the PTC of the ribosome as the binding site of agelastatin A. Initial studies using next-generation dimethylsulfate footprinting experiments revealed ribosomal residues that were likely interacting with the natural product, tracing back the agelastatin A binding site to the PTC. By leveraging their past X-ray crystal structure data of the ribosome complexed to known PTC binders (Garreau de Loubresse et al., 2014), computational docking studies then predicted the molecular interactions between agelastatin A and the PTC. In particular, this suggested the presence of hydrogen bonds between the D-ring dihydropyrazinone of agelastatin A and neighboring residues, as well as  $\pi$ - $\pi$  interactions between the A-ring pyrrole and nearby aromatic bases. Ultimately, McClary et al. corroborated these in silico predictions by obtaining an X-ray crystal structure of the ribosome-agelastatin A complex. Their structure identified additional binding interactions such as hydrogen bonding between the C5-hydroxyl group and uracil 2873, as well as a noteworthy halogen- $\pi$  interaction between the C13-bromine atom with uracil 2875.

The X-ray crystal structure data were largely consistent with past SAR data (Jouanneau et al., 2016; Han et al., 2013). By comparing the inhibitory effects of different members of the agelastatin family, the authors found that perturbation of the putative D-ring hydrogen-bonding interactions resulted in a significant loss in activity. Additionally, replacement of the pyrrole A-ring hydrogens at C14 and C15 positions with larger atoms led to a significant loss in activity, most likely due to increased steric repulsion between the pyrrole ring and its neighboring residues that could preclude  $\pi$ - $\pi$  stacking



**Figure 1. Structure of Agelastatin A**  
Agelastatin A is a natural product first isolated from a sea sponge, *Agelas dendromorpha*. It contains a characteristic tetracyclic structure with a halogenated pyrrole ring.

interactions. By contrast, installation of electron-withdrawing substituents that could enhance  $\pi$ - $\pi$  stacking improved the potency of the molecule, lending further credence to their binding model.

In addition to elucidating the molecular interactions between agelastatin A and the ribosome PTC, McClary et al. modeled conformational changes within the PTC induced by the natural product. By comparing the agelastatin-bound ribosome to the unbound state, they were able to identify key residues that underwent conformational reorganization to accommodate the natural product. Such reorganization is not unique to agelastatin A. Other ribosomal inhibitors that are

structurally dissimilar have been shown to bind to the same nucleotides in the PTC as agelastatin A (Garreau de Loubresse et al., 2014), but all three compounds induce distinct conformational changes in the PTC. Taken together, these results underscore the potential plasticity of the PTC binding pocket; whether such information can be synthesized together to develop improved inhibitors remains an exciting question.

Here, McClary et al. firmly establish the eukaryotic ribosome as a cellular target of agelastatin A, resolving the over-two-decade mystery surrounding the molecule's mechanism of action. A wide range of structural and modeling studies were used to narrow down the molecule's binding site to the PTC, identifying key binding interactions that were validated through synthetic agelastatin derivatives. However, some important questions remain. In particular, how and whether global inhibition of protein synthesis leads to the myriad of biological activities exhibited by agelastatin A remains to be fully fleshed out. In summary, McClary et al. provide an exemplar of small-molecule target identification and pave the path for the development of agelastatin analogs for potential therapeutic purposes. More broadly, this current and past body of work from the authors have provided

a critical window into understanding how small molecules can bind the ribosome and inhibit translation, revealing fine detail of the ribosome's structure and function.

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## HSP60 Takes a Hit: Inhibition of Mitochondrial Protein Folding

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In this issue of *Cell Chemical Biology*, Wiechmann et al. (2017) identify mitochondrial chaperonin HSP60 as a direct target of myrtoicommulone (MC), a nonprenylated acylphloroglucinol that is well known for its apoptotic activity in cancer cells. The authors propose MC as a chemical probe to study HSP60 biology and a potential chemotherapeutic agent in treating cancer and other HSP60-associated diseases.

Mitochondria have taken center stage in biomedical research aimed at discovering the molecular mechanisms underlying

human disease and aging and potential therapeutics to cure or manage human pathologies. These organelles and their

resident proteins have emerged as crucial players that orchestrate cellular energetics, calcium and iron homeostasis,