From plant physiology to pharmacology: fusicoccin leaves the leaves

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Fusicoccin (FC, figure 1) is a phytotoxic glycosylated diterpene produced by the fungus *Phomopsis amygdali*, a pathogen of almond and peach plants [1]. Widespread interest in this molecule started when it was discovered that it is able to influence a several fundamental processes in plants, including stomata opening, cell expansion and seed germination, which are dependent on the activation of the plasma membrane H^+-ATPase, the electrogenic proton pump that provides the driving force for ion and solute transport and for cell turgor maintenance. Molecular studies carried out in the last twenty years clarified details of the mechanism of proton pump stimulation, which involves the FC-mediated stabilization of the interaction between the H^+-ATPase and a class of regulatory polypeptides, named 14-3-3 proteins [1].

14-3-3 proteins are ubiquitous regulators occurring as numerous isoforms in eukaryotic organisms [2]. These proteins are involved in the control of pivotal physiological processes, such as cell cycle progression, apoptosis, cellular trafficking, and gene transcription. In plants, they also accomplish further peculiar functions, such as regulation of nitrogen and carbon metabolism, ion transport, hormone and light signaling [3]. 14-3-3 proteins exert their effects by binding to phosphorylated client proteins, thereby modulating their sub-cellular localization, enzymatic activity, turn-over, or their ability to associate with other proteins. The structure of 14-3-3 proteins has been clarified by X-ray crystallography (figure 2). Each monomer comprises nine anti-parallel α-helices and binds through its *N*-terminus a second monomer to assemble the functional dimeric protein. The 14-3-3 dimer has a characteristic W-like shape, with a conserved internal surface and a more variable external surface. The internal surface contains a conserved amphipathic cavity, which is responsible for the interaction with the phosphorylated target. Analysis of the phosphorylated binding sequences of 14-3-3 clients revealed that 14-3-3 proteins recognize pSer (p=phosphate) and pThr-containing motifs called mode I (RSX(pS/pT)XP and mode II (RXY/FX(pS/pT)XP respectively [2].

FC do not bind *per se* to 14-3-3 proteins, but rather to the H^+-ATPase/14-3-3 complex [3]. The toxin irreversibly stabilizes the interaction, which displaces the autoinhibitory C-terminal domain, strongly stimulating the activity of the enzyme. This finding, which was rather surprising since no canonical mode I or II binding motifs are on the proton pump, led to the discovery of a novel binding site, the mode III motif, located at the extreme C terminus of the enzyme, that is generated by the phosphorylation of a conserved Thr residue [3]. The resolution of the crystal structure of the ternary complex between FC, 14-3-3 proteins and the phosphorylated C-terminal peptide of the H^+-ATPase shed light on the molecular basis of toxin binding (Figure 2B). The phosphopeptide is accommodated by the amphipathic groove that 14-3-3 proteins typically use to bind their targets, while FC is arranged next to the C terminus of the phosphopeptide, making contact both with the peptide and the 14-3-3 groove. Isothermal titration calorimetry and surface plasmon resonance experiments clarified that FC binds 14-3-3 proteins with very low affinity, whereas the peptide and the toxin reciprocally enhance, by approximately two orders of magnitude, their respective binding affinities, bringing about a strong stabilization of the ternary complex [3].

The occurrence of FC binding sites was initially reported to be restricted to higher plants. Nevertheless, the identification of the FC receptor as a complex between 14-3-3 proteins and the H^+-ATPase client raised the question as to whether other receptors exist, since many 14-3-3 protein targets have been described. The resolution of the structure of the ternary complex between FC, 14-3-3 proteins and the H^+-ATPase provided the rationale to clarify that the stabilizing effect of FC occurs only with mode III clients, whereas the toxin is ineffective with mode I and II targets, which account for most 14-3-3-interacting proteins. Nevertheless, even though to a reduced extent as compared to mode I and II clients, different mode III 14-3-3 clients have so far been identified in plants and animals, a fact that leaves open the question of the occurrence of further FC receptors and prompted studies to search novel FC targets. The first piece of evidence that FC can be a general regulator of the interactions between 14-3-3 proteins and mode III targets has been obtained in human platelets, where it has been shown that the toxin stimulates the 14-3-3 association to the glycoprotein Ibα [4]. This protein is part of GPIb-IX-V, a complex that mediates the initial adhesion of circulating platelets to the sub-endothelial von Willebrand Factor. This, in turn, results in platelet activation and consequent aggregation [4]. A similar mechanism involving FC stabilization of the binding of 14-3-3 proteins to a mode III client has been discovered for the estrogen receptor α (ERα) and for the cystic fibrosis transmembrane conductance regulator (CFTR), the anion and bicarbonate transporter implicated in cystic fibrosis [5]. Very recently, it has been demonstrated that FC stimulates damaged axon growth *in vitro* and regeneration *in vivo* of rat cortical embryonic neurons [6]. The effect is mediated by the stress response regulator GCN, which forms a complex with 14-3-3 proteins which is stabilized by FC, thereby resulting in neurite outgrowth and regeneration [6].

The structural requisites of mode III motifs that allow FC binding and the formation of the FC/14-3-3/target ternary complex has been elucidated by bioinformatics and in vitro by Isothermal titration calorimetry (ITC) analysis. These studied revealed that the FC effect is solely dependent on the physiochemical properties of the residue in position +1 with respect to the pSer/pThr. In general, the interaction is favored with hydrophobic side chains of the C-terminal amino acid. However, steric hindrance and/or limited plasticity of cyclic rings can obstruct the accommodation of FC in the 14-3-3-binding cavity, thereby hampering the assembly of the ternary complex [3].

The question raised from recent research is whether FC could a tool in pharmacological research, since several 14-3-3 clients are involved in a wide array of physiological and/or pathological processes. In fact, whereas the development of small-molecule inhibitors of 14-3-3/client interaction is a relevant issue in pharmacology, in alternative cases drug-mediated stabilization of 14-3-3/client interaction may be a more convenient approach. In this respect, the peculiar nature of FC as a cell-permeable stabilizer of 14-3-3 interactions with mode III targets certainly represents a promising starting point for research aimed to deepen the knowledge about the biochemical consequences of protein-protein interaction modulation, as well as to develop new therapeutic agents derived from FC.

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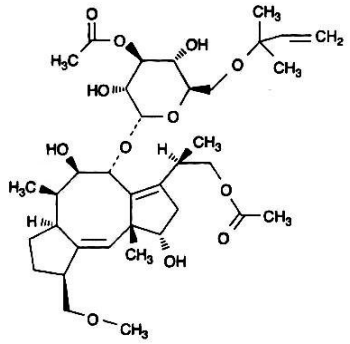

Figure 1. Structure of FC

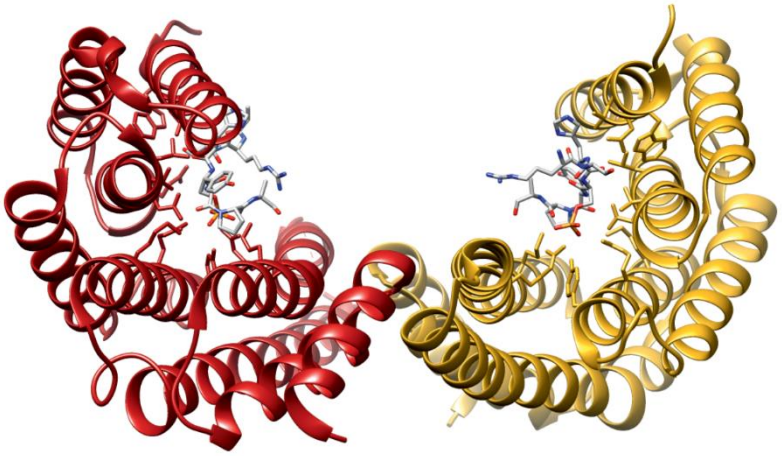

Figure 2. Structure of 14-3-3 proteins. Ribbon plot of the human 14-3-3ζ dimer (PDB 1QJB), showing the two monomers (red and yellow ribbon, respectively) complexed with the mode I Raf-1 phosphopeptide, which is bound in an extended conformation to the amphipathic groove of each monomer.

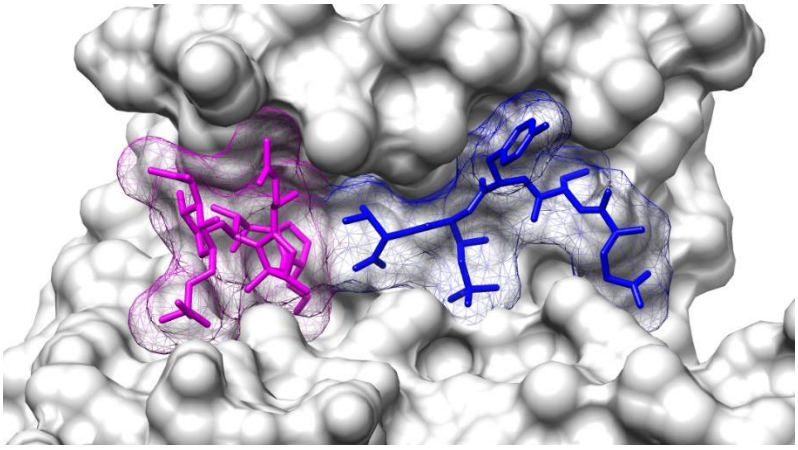

Figure 3. Crystal structure of the FC/phosphopeptide/14-3-3 ternary complex. 14-3-3, grey surface; FC, magenta; H^+-ATPase pentapeptide QSYpTV, blue.