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# The mechanosensitive APJ internalization via clathrin-mediated endocytosis: A new molecular mechanism of cardiac hypertrophy

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ABSTRACT

The G protein-coupled receptor APJ elicits cellular response to diverse extracellular stimulus. Accumulating evidence reveals that APJ receptor plays a prominent role in the cardiomyocyte adapting to hypertrophic stimulation. At present, it remains obscure that the regulatory mechanism of APJ receptor in myocardial hypertrophy. The natural endogenous ligands apelin and Elabela as well as agonists maintain high affinity for the APJ receptor and drive its internalization. Ligand-activated receptor internalization is mainly performed by clathrin-mediated endocytic pathway. Simultaneously, clathrin-mediated endocytosis takes participate in the occurrence and development of cardiac hypertrophy. In this study, we hypothesize that natural ligands and agonists induce the mechanosensitive APJ internalization via clathrin-mediated endocytosis. APJ internalization may contribute to the development of cardiac hypertrophy. The mechanosensitive APJ internalization via clathrin-mediated endocytosis may be a new molecular mechanism of cardiac hypertrophy.



## Introduction

The heart responds to various neurohormonal factors or persistent increased hemodynamic stress through a series of compensatory process, including changes in chamber volume, systolic contraction, diastolic relaxation, heart rate and muscle mass. Pathological cardiac hypertrophy is commonly stimulated by stress or induced by some diseases, such as hypertension, valvular heart disease, myocardial infarction or neurohormones [1,2]. Although some advance therapies are existed, more effective therapeutic strategy for cardiac hypertrophy still need further investigation.

Currently, a large number of studies indicate that APJ receptor plays an important role in cardiac hypertrophy. APJ, which is consisting of 377 amino acids, is a G protein-coupled receptor (GPCR) with seven transmembrane domains. The APJ gene sequence is significantly homologous to 54% with angiotensin II receptor type-1 (AT1) [3]. The endogenous ligand apelin and Elabela binding with APJ receptor have a variety of biological function for cardiovascular system [4–12]. APJ has been shown to be expressed in various tissues especially for the myocardial cells [13]. The APJ likely serve as mechanosensitive receptor, which is essential for the occurrence and development of cardiac hypertrophy [14–16]. Thus, it is necessary to elicit that the pathophysiological profile and regulatory mechanism for APJ signaling in cardiac hypertrophy.

Clathrin-mediated endocytosis (CME, also known as receptor-mediated endocytosis) is main pathway for extracellular macromolecular ligands gaining access into the intracellular environment. The process of ligands bound to a transmembrane receptor at the cell surface is concentrated in certain parts of the plasma membrane (named coated pits), giving rise to endocytic vesicles that are then pinched off inside the cell [17] (Fig.1). There are several classical receptor–ligand systems that are involved in CME, such as insulin–insulin receptor and platelet derived growth factor–platelet derived growth factor receptor (PDGF–PDGFR) [18,19]. Moreover, GPCR internalization usually is require for clathrin-mediated endocytic pathway, which is relevant to maintain cardiac function and contribute to the development of cardiac hypertrophy [20].

In recently, some studies describe that natural ligands (apelin and Elabela) and agonists may stimulate APJ receptor internalization. However, there is no document regarding that the molecular mechanism of APJ internalization and the role of APJ internalization in cardiac hypertrophy. In this hypothesis we propose that the mechanosensitive APJ internalization though clathrin-mediated endocytosis contributes to the development of cardiac hypertrophy. The mechanosensitive APJ internalization via clathrin-mediated endocytosis may be a new molecular mechanism of cardiac hypertrophy.

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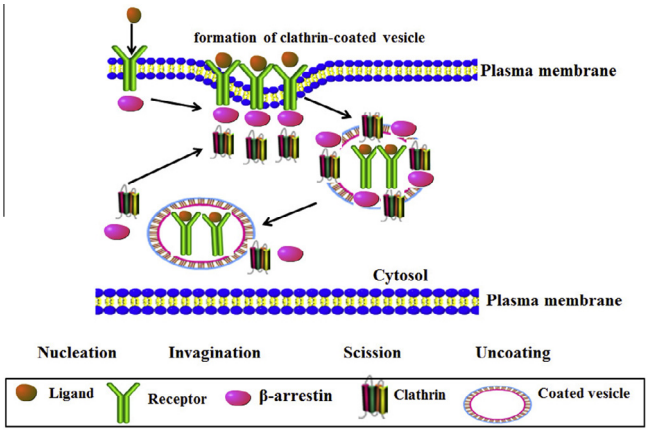


**Fig. 1.** The clathrin-mediated endocytosis. The four main steps of clathrin-dependent endocytosis are shown. (1) Nucleation, where ligands and agonists binding with receptor are to be internalized and gathering into the forming pit; (2) invagination, where membrane receptor recognizes the ligand and forms a polyhedral cage around the coat vesicle by clathrin via β-arrestin-dependent manner; (3) scission, where the clathrin-coated vesicle is separate from the plasma membrane; (4) uncoating, where the clathrin-coat vesicle is removed and the nascent vesicle is freed.

## Basis for the hypothesis

### *The properties of APJ receptor in cardiac hypertrophy*

The APJ receptor has an emerging role in cardiac properties. Recently, Scimia et.al have reported that administration of apelin delays the progression to cardiac hypertrophy in response to pressure overload via Gαi-protein pathway, apelin binding to APJ receptor plays a protective cardiac hypertrophic response [14–16]. Moreover, the over-expression of apelin ameliorates the development of cardiac hypertrophy [21–28]. Whereas the activation of APJ promotes stretch-induced cardiomyocyte cell size, protein content and the expressions of pro-hypertrophic-associated factors which are reversed by β-arrestin knockdown, indicating stretch/APJ signal is sufficient to trigger pressure overload-induced cardiac hypertrophy in β-arrestin-dependent manner. Coincidentally, the knockout (APJ-KO) mice diminishes cardiomyocyte size and reduces heart weight/body weight (HW/BW) ratio [14]. Our laboratory have confirmed that the APJ acts as a static pressure sensor receptor to provoke cardiomyocyte hypertrophy thought the activation of PI3K-autophagy pathway [29] Therefore, APJ serves as the mechanosensitive receptor, which represents an important linking for pathological cardiac hypertrophy and may be a potential therapeutic target.

### *Clathrin-mediated endocytosis and cardiac hypertrophy*

The clathrin-mediated endocytic pathway is essential for maintaining cardiac function and plays essential role in cardiac hypertrophy. Indeed, endocytosis has been involved in keeping the morphological characterization of mouse cardiac cell [30]. The upregulation of myocardial Rab1GTPase, which may be regulating vesicle transport during endocytosis, is sufficient to trigger cardiac hypertrophy [31]. In adult feline cardiomyocytes, integrin-interacting RGD peptide interaction with β3 integrin, which activates S6K1, MEK/ERK and PI3K/mTOR via endocytosis, contributes to the hypertrophic growth [32]. Moreover, interference with endocytosis by Concanavalin A in neonatal rat cardiomyocyte blocks the β-adrenergic receptor (β-AR)-mediated cardiac hypertrophy though PI3K and Akt signal pathway [33,34]. Along these same lines, transmembrane transport of Gαq protein carboxyl terminus imitation polypeptide-27 (GCIP-27) and sympathetic norepinephrine of α (2A)-adrenoceptors-mediated endocytosis are involved in cardiac hypertrophy [35,36]. The steroid hormone ouabain induces endocytosis of the Na/K-ATPase, which depends on clathrin-coated pits formation, thus contributing to the formation of cardiac hypertrophy [37]. Consistently, reactive oxygen species (ROS) may cross with clathrin-mediated endocytosis of membrane receptor Na/K-ATPase, leading to the activation of hydrogen peroxide-induced hypertrophy in cardiac myocytes [38]. Altogether, clathrin-mediated endocytosis participates in the occurrence and development of cardiac hypertrophy.

## The molecular characterization of mechanosensitive APJ internalization

There are two natural endogenous ligands of APJ receptor named Apelin and Elabela (Todder) [11,12]. Besides that, some bioavailable agonists for APJ receptor had also been developed, such as E339-3D6 and MM07 [39–41]. Both endogenous ligands and agonists behave as high affinity and induce the mechanosensitive APJ internalization [42].

Apelin is one of the natural endogenous ligands for APJ receptor. The molecular isoforms of apelin naturally occurring *in vivo* are apelin-36, apelin-13 and apelin-17. The pyroglutamyl form of apelin-13 (pE13F) usually used *in vitro*. These compounds have high affinity for the APJ receptor. In transfected Chinese hamster ovary (CHO) cells, C-terminus domain of K17F and pE13F binding with Trp (259) and Phe (255) residues of APJ receptor drives its internalization. Moreover, the identified phosphorylated 348 serine in the C terminus of APJ plays a crucial role in promoting its internalization though β-arrestin-dependent ERK1/2 signaling, but not Gα (i)-protein coupling [43–50]. Herein, apelin-13 and apelin-36 also result in APJ rapidly internalization in dose-dependent way. The internalized APJ induced by apelin-13 dissociate from β-arrestin1 and then recycle to the cell surface via Rab4-dependent manner, while APJ receptors internalized stimulated by apelin-36 associated with β-arrestin1 but still remained in the cytoplasm by Rab4 [45,51,52]. E339-3D6 and MM07, which are two biased agonists of APJ receptor, also behave as high affinity and promote the APJ internalization [39–41]. Thus, the natural ligands and APJ agonists are crucial determinants for triggering mechanosensitive APJ rapidly internalization and exert a widely of pathophysiological profile (Table.1).

## Evaluation for hypothesis: the mechanosensitive APJ internalization via clathrin-mediated endocytosis in cardiac hypertrophy

As well known that β-Adrenoceptor internalization upon ligand binding though clathrin-mediated endocytosis, plays diverse roles in the stimulation of hypertrophic factors [53,54]. In above-mentioned, APJ receptor is also able to undergo rapid internalization on plasma membrane upon endogenous ligands and agonists-induced activation. Indeed, apelin and K13F are potent inducers of APJ internalization which may be mediated by clathrin-coated pits. E339-3D6 also increases the APJ endocytosis though clathrin-dependent manner [39,44,50,51]. An outline of the potential signaling cascade involved in cardiac hypertrophy activation by mechanosensitive APJ internalization though clathrin-mediated endocytosis.

In this study, we hypothesize that the mechanosensitive APJ internalization is probably mediated by clathrin-mediated endocytic pathway. Furthermore, APJ internalization may contribute to the development of cardiac hypertrophy. The mechanosensitive APJ internalization via clathrin-mediated endocytosis may be a new molecular mechanism of cardiac hypertrophy (Fig. 2).