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# Endocytosis of hERG Is Clathrin-Independent and Involves Arf6

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**Abstract**

The hERG potassium channel is critical for repolarisation of the cardiac action potential. Reduced expression of hERG at the plasma membrane, whether caused by hereditary mutations or drugs, results in long QT syndrome and increases the risk of ventricular arrhythmias. Thus, it is of fundamental importance to understand how the density of this channel at the plasma membrane is regulated. We used antibodies to an extracellular native or engineered epitope, in conjunction with immunofluorescence and ELISA, to investigate the mechanism of hERG endocytosis in recombinant cells and validated the findings in rat neonatal cardiac myocytes. The data reveal that this channel undergoes rapid internalisation, which is inhibited by neither dynasore, an inhibitor of dynamin, nor a dominant negative construct of Rab5a, into endosomes that are largely devoid of the transferrin receptor. These results support a clathrin-independent mechanism of endocytosis and exclude involvement of dynamin-dependent caveolin and RhoA mechanisms. In agreement, internalised hERG displayed marked overlap with glycosylphosphatidylinositol-anchored GFP, a clathrin-independent cargo. Endocytosis was significantly affected by cholesterol extraction with methyl-β-cyclodextrin and inhibition of Arf6 function with dominant negative Arf6-T27N-eGFP. Taken together, we conclude that hERG undergoes clathrin-independent endocytosis via a mechanism involving Arf6.

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## Introduction

The hERG (human ether-a-go-go related gene) potassium channel (Kv11.1), encoded by the *KCNH2* gene, underlies the rapidly activating delayed rectifier $K^+$ current ($I_{Kr}$). This forms a crucial component of the repolarisation phase of the cardiac action potential and a reduction in its activity is associated with prolongation of the QT interval in the electrocardiogram (long QT syndrome 2; LQT2), which increases the risk of ventricular fibrillations and sudden death [1,2]. This aberration in the electrical activity of the heart has been identified for ~300 inherited mutations [2,3] and linked to a wide range of drugs [4,5], leading to their removal from the market and failure of new drugs in preclinical testing. Loss of function results from reducing the activity and/or the cell surface density of hERG.

Surface levels are determined by the balance between channel insertion into the cell membrane, from forward (biosynthetic) trafficking and recycling of endocytic channels back to the surface, and channel removal by endocytosis. Reducing forward trafficking represents one mechanism by which hERG surface density is decreased. LQT2 mutations and drugs can cause misfolding of newly synthesised channels, resulting in their retention in the ER, polyubiquitination and degradation by the cytosolic proteasomes [6,7]. Alternatively, endocytic trafficking of hERG can be disrupted, altering channel removal from the surface, recycling back to the cell membrane and/or targeting for endosomal degradation. This mechanism is less established, but has been implicated in the impact of certain drugs [8,9] and pathophysiological conditions such as hypokalaemia [10,11] and hyperglycaemia [12,13]. Therefore it is important that we understand the fate of hERG after it is inserted in the plasma membrane, something that has so far received little attention.

Most membrane proteins are removed from the surface by endocytosis and are then either recycled back to the plasma membrane or undergo degradation [14,15]. Unlike biosynthetic delivery, which is slow (hours) [16,17], endosomal trafficking events can occur on a rapid time scale (minutes) [18,19]. Thus,