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Review

Transitioning pharmacoperones to therapeutic use: *In vivo* proof-of-principle and design of high throughput screens

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ABSTRACT

A pharmacoperone (from "pharmacological chaperone") is a small molecule that enters cells and serves as molecular scaffolding in order to cause otherwise-misfolded mutant proteins to fold and route correctly within the cell. Pharmacoperones have broad therapeutic applicability since a large number of diseases have their genesis in the misfolding of proteins and resultant misrouting within the cell. Misrouting may result in loss-of-function and, potentially, the accumulation of defective mutants in cellular compartments. Most known pharmacoperones were initially derived from receptor antagonist screens and, for this reason, present a complex pharmacology, although these are highly target specific. In this summary, we describe efforts to produce high throughput screens that identify these molecules from chemical libraries as well as a mouse model which provides proof-of-principle for *in vivo* protein rescue using existing pharmacoperones.

Contents

Abbreviations: GPCRs, G protein coupled receptors; V2R, vasopressin type 2 receptor; GnRHR, gonadotropin releasing hormone receptor; QCS, quality control system; ER, endoplasmic reticulum; PM, plasma membrane; GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; HH, hypogonadotropic hypogonadism; NDI, nephrogenic diabetes insipidus; cAMP, adenosine monophosphate; "Dox", doxycycline; S/B, signal-to-background ratio; TMS, transmembrane segment; IVF, *in vitro fertilization*; StAR, steroidogenic acute regulatory protein.

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1. Introduction

For more than 20 years, there has been interest in gene therapy as a means to correct mutational disease. Issues related to the integration of therapeutic DNA into the genome, immune responses, technical problems with vectors (toxicity, immune, inflammatory responses, gene control and targeting issues), chances of inducing tumors, (insertional mutagenesis) and other problems, have made it challenging to reduce this approach to practice, however.

Correcting the folding of misfolded protein mutants and restoring them to function (with pharmacoperone drugs) is a potential alternative to inserting correctly folding proteins by gene therapy (Fig. 1). This approach is generally referred to as "rescuing" the protein. It is likely that valuable drugs reside in chemical libraries, yet have been missed, since screening approaches that rely on identification of agonists and antagonists may have failed to identify existing pharmacoperones. There are several advantages to using pharmacoperone drugs among these advantages are the ability to restore misfolded proteins to function without leaving residual non-functional proteins in other cellular compartments.

Protein rescue with agents that correct folding errors potentially applies to a diverse and vast array of human diseases that result from misfolding or instability of receptors, ion channels, enzymes and other proteins. These include cystic fibrosis [1–5], hypogonadotropic hypogonadism (HH) [6,7], nephrogenic diabetes insipidus [8–10], retinitis pigmentosa [11], hypercholesterolemia [12], cataracts [13], neurodegenerative diseases (Alzheimer's, Huntington's and Parkinson's, [14–18]), cancer [19], α1 trypsin deficiency and lysosomal storage disease [20,21], mucopolysaccharidosis type IIIC [22] and many others. One could envision drugs given in a prophylactic manner (in vitamins, for example) that prevent the misfolding that leads to neurodegenerative disorders (Alzheimer's (misfolded amyloid), [23]) Parkinson's (misfolded α-synuclein) and cataracts (misfolded lens crystalline). In this regard, diseases may be prevented before clinical signs present. In the case of certain proteins (*e.g.* the GnRHR, V2R and rhodopsin), this approach has succeeded with a striking number of different mutants [24] supporting the view that pharmacoperones will become powerful weapons in our therapeutic arsenal.

A large number of proteins of different classes and structures that have been rescued and restored to function in cell cultures is also suggestive of the broad applicability of this approach [25–39].

Our efforts have focused on G protein coupled receptors (GPCRs), which include the vasopressin type 2 receptor (V2R) and gonadotropin releasing hormone receptor (GnRHR). GPCRs comprise the largest family of validated drug targets – 35% to 50% of approved drugs derive their benefits by selectively targeting this family.

2. Mechanism of pharmacoperone action

GPCRs are subjected to a stringent quality control system (QCS) in the endoplasmic reticulum (ER); this system consists of both, protein chaperones that retain misfolded proteins and enzyme-like proteins that catalyze the folding process. The QCS (consisting of endogenous chaperone proteins and other factors) assesses

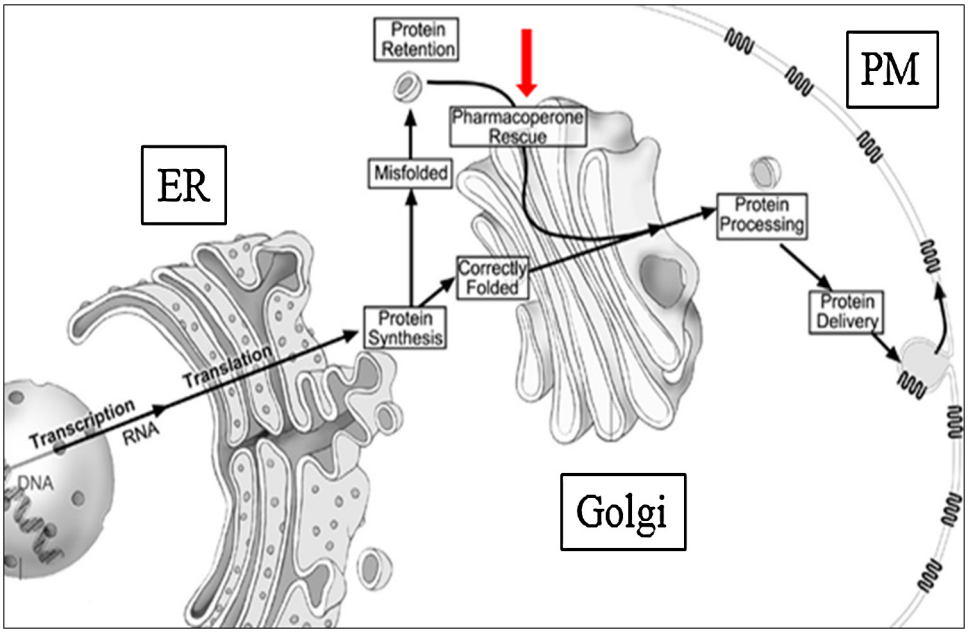

Fig. 1. The canonical pathway of protein translation from mRNA, emphasizing that misfolded proteins are retained in the cell (ER and elsewhere) and can be rescued by target specific pharmacoperone drugs (red arrow) that correct misfolding and restore function. (For interpretation of the references to color in text, the reader is referred to the web version of this article.)

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