Minireview

New insights on the functions of the guanylyl cyclase receptors

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Abstract The discovery of at least 29 genes encoding putative guanylyl cyclases in *Caenorhabditis elegans* has raised the question as to whether there are numerous receptors yet to be discovered in the mammal. The nematode, however, not only seems ideal to study guanylyl cyclase receptor localization and function, given the large variety of isoforms, but also leads to possible identification of ligands for orphan guanylyl cyclases by the use of genetic and behavioral assays. A recent powerful approach to describe the function of different guanylyl cyclase isoforms in mammals has been the disruption of the corresponding genes in the mouse. A salt resistant elevation of blood pressure, which corresponds to the phenotype of 50% of all human patients with essential hypertension, is observed in mice lacking the GC-A-receptor. Mice missing the GC-C receptor have been shown to be resistant to STa, an *E. coli* heat-stable enterotoxin, which is largely responsible for travellers diarrhea in adults and mortality due to diarrhea in infants.

Key words: Guanylyl cyclase; Cyclic GMP; *Caenorhabditis elegans*; Green fluorescent protein; Neuron; Sensory; Odorant; Pheromone; Gene disruption; Hypertension; Diarrhea

1. Introduction

Following the discovery of cyclic GMP in 1963 [1], guanylyl cyclase activity was found in both the particulate and soluble fractions of various tissue homogenates [2–4]. It was assumed that the Second Messenger Hypothesis for cAMP established by Sutherland and coworkers [5] would also apply for the regulation of cGMP. Therefore, it was speculated that hormones which would elevate cyclic GMP in intact cells also would activate guanylyl cyclase in broken cell preparations; however, initial experiments met with failure. It eventually became evident that guanylyl cyclases were different from adenylyl cyclases in that the ligand binding (receptor) and effector (cyclase) domain reside within the same molecule. Over the last 20 years both the membrane and soluble forms have been shown to be directly activated by various ligands in broken cell as well as in purified enzyme preparations.

The membrane and soluble forms of guanylyl cyclase possess unique primary structures that appear consistent for each of the subfamily members across wide evolutionary distances; these structures are summarized in Fig. 1.

In the mammal there are six membrane forms (GC-A-F) and two α (α_1, α_2) and two β (β_1, β_2) subunits of the heterodimeric soluble form identified [6]. For three of the six membrane forms (GC-A-C) ligands have been identified, which fall into two families: the natriuretic peptides, and the heat-stable enterotoxins of *E. coli*, for which the endogenous homologs have been suggested as guanylin/uroguanylin [7,8]. The best characterized form of the soluble guanylyl cyclase, the α_1/β_1-heterodimer, is stimulated by binding of nitric oxide to a prosthetic heme group [9–11]. Recently another gaseous molecule, carbon monoxide has been proposed as a ligand for soluble guanylyl cyclase [12,13].

2. Functions of guanylyl cyclase receptors

2.1. Orphan receptors

There are different approaches to gain insight into the function of the various guanylyl cyclase receptors, including the discovery of ligands for the orphan receptors. In the mammal, two orphan receptors exist in the retina (GC-E, GC-F; [14–16]), and one in the olfactory neuroepithelium (GC-D; [17]). GC-D is expressed in a subset of neurons that appear unique compared to the majority of sensory neurons found in the nose [18]. Determination of the function of GC-D may require the identification of its ligand, but since the ligand may be a pheromone/odorant, this likely represents a daunting task, given a ligand has not yet been identified for one of the mammalian 7-transmembrane, putative odorant receptors. In *Caenorhabditis elegans*, in contrast, the apparent 7-transmembrane receptor for diacetyl has been discovered [19]. We initiated studies on the nematode (*C. elegans*) to determine whether guanylyl cyclase receptors were present, and if so, whether any existed within sensory neurons. A *C. elegans* cDNA library initially yielded a single clone encoding a putative membrane form of guanylyl cyclase [20]. The cDNA was then expressed in COS-7 cells to demonstrate that the clone actually encoded a protein with guanylyl cyclase activity. Subsequent searches of the *C. elegans* genome database revealed the presence of at least 29 gene products that appeared to encode guanylyl cyclases. By the use of green fluorescent protein (GFP) as a reporter gene product, it was shown that many of these orphan guanylyl cyclases are expressed in distinct sensory neurons (Fig. 2; [21]). In some cases, even neurons previously thought to be symmetrical, expressed orphan guanylyl cyclase receptors on only the right or the left side [21]. These results raise the question of whether guanylyl cyclase receptors play a major role in odorant/pheromone signaling and/or whether they function in other neuronal processes. The fact that the nematode genome is about 1/30 the size of the mammalian genome also raises the question of whether there are many more guanylyl cyclase receptors still to be discovered in the mammal.

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Abbreviations: GC-A-F, guanylyl cyclase receptors-A-F; ANP, BNP and CNP, A-type, B-type and C-type natriuretic peptide; GFP, green fluorescent protein

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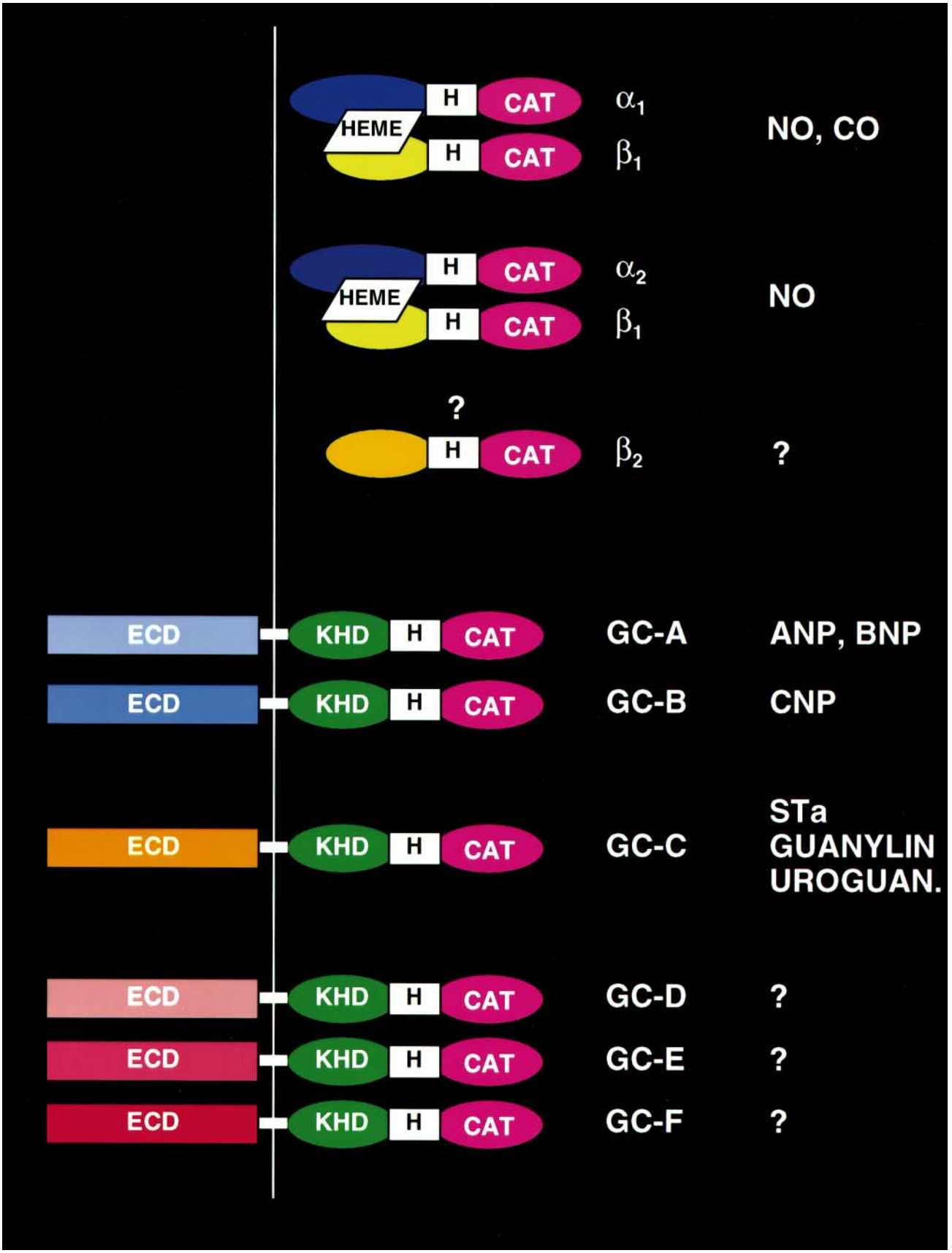
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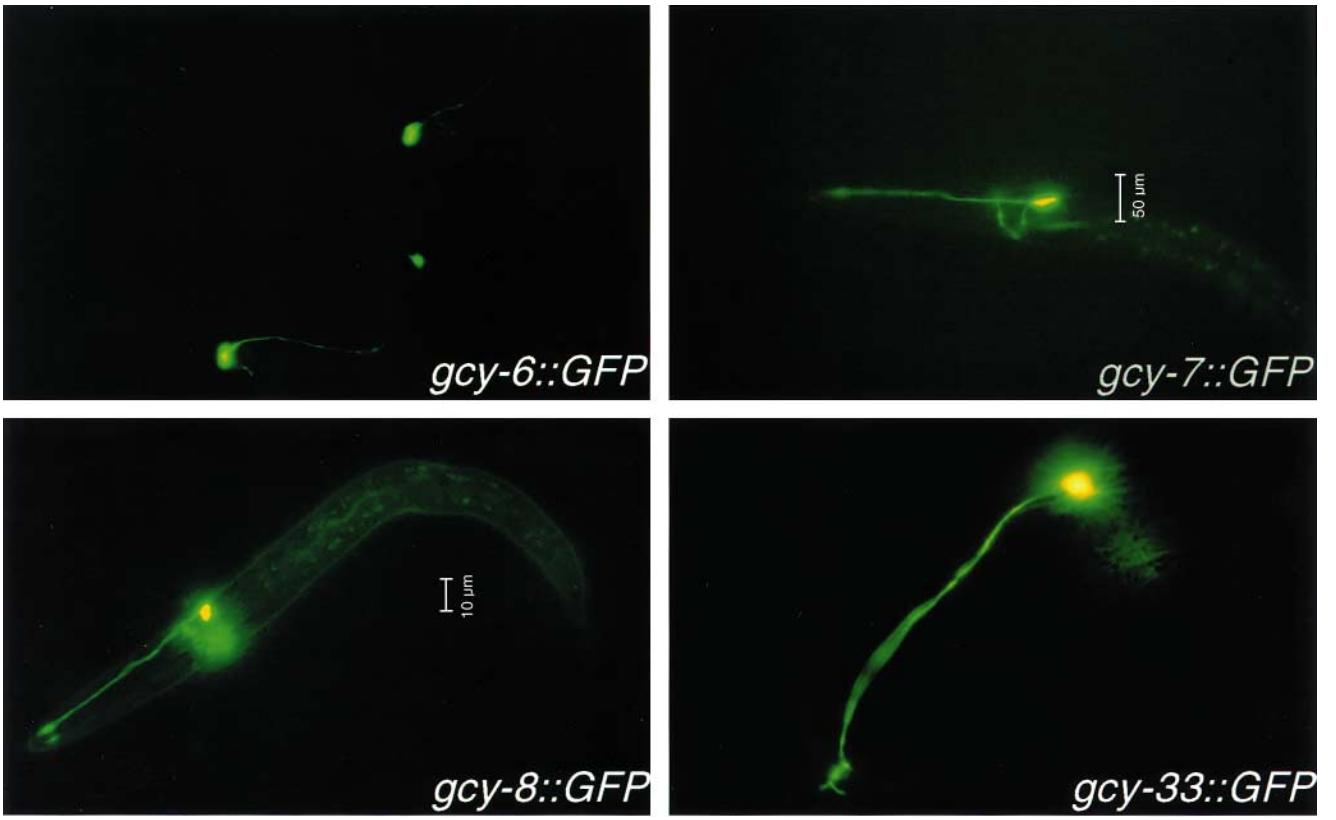

Fig. 2. Expression of promoter::green fluorescent protein (GFP) in *C. elegans*. The promoters for membrane guanylyl cyclases GCY-6, GCY-7, GCY-8, or GCY-33 were used to drive the expression of GFP. The GCY-33 product is predicted to be a soluble form of guanylyl cyclase, most closely resembling the β subunit from mammals. The bright green images presumably represent sites of expression of each of the orphan guanylyl cyclases in different sensory neurons.

2.2. Dominant negative mutants

Both the cytoplasmic and membrane forms of guanylyl cyclase appear to exist as dimers or higher ordered structures, and thus various mutations have led to the formation of dominant negative subunits. In some cases these have been mutations which inactivate cyclase catalytic activity [22,23] while in other cases they have prevented ligand stimulation [24]. Although these mutant subunits have yet to be successfully used as reagents to define the importance of any particular cyclase signaling pathway, their overexpression in transgenic animals or in virus-infected tissues or cells, in principal, could help define the functions of various cyclase receptor signaling pathways. Furthermore, that a single point mutation in one subunit can inactivate the cyclase suggests that a single substrate (GTP) molecule is bound through shared subunit binding sites. Thus, it has been proposed that a dimer is the minimal catalytic unit of either adenylyl or guanylyl cyclases [10,11].

2.3. Constitutively active mutants

There are two types of general mutations which appear to lead to constitutively active membrane forms of guanylyl cyclase. The first is deletion of the protein kinase-like domain [25], and the second is mutation of $Glu^{974}Ala$, both in GC-A [26]. Since GC-A lacking the protein kinase-like domain is active and no longer regulated by ANP/ATP, it has been suggested that the protein kinase domain normally serves to repress the activity of the cyclase catalytic domain and that ANP/ATP relieve this inhibition [25]. The point mutation (E974A) results in about a 14-fold increase in GC-A activity, which is observed even in the presence of Mn^{2+}/Triton X-100 [26]. The elevated cyclase activity is not explained by relief of protein kinase domain inhibition since a mutant protein lacking the protein kinase-like domain also expresses high cyclase activity. Unlike the protein kinase-like domain deletion mutant, the point mutant (E974A) also converts the kinetic pat-

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Fig. 1. The predicted primary structures of soluble and membrane guanylyl cyclases. All guanylyl cyclase isoforms contain a carboxyl terminal consensus catalytic domain (CAT), which is homologous to the catalytic domain of adenylyl cyclase. Another common feature of all guanylyl cyclases is an amphipathic, predicted α-helical dimerization domain (hinge region; H) The soluble forms appear to exist only as heterodimers, and demonstrate no catalytic activity unless the dimer is formed [11]. While the β_1 subunit dimerizes with the α_1 and α_2 subunits, a partner for the β_2 subunit has not yet been identified. The amino terminus likely binds heme [24], the site of interaction with the ligands NO and CO. The membrane forms appear to contain a single transmembrane segment that separates an extracellular ligand-binding domain (ECD) from intracellular protein kinase-like (KHD), hinge (H) and cyclase catalytic domains (CAT). The membrane forms appear to exist as homodimers or higher ordered homomers [32].

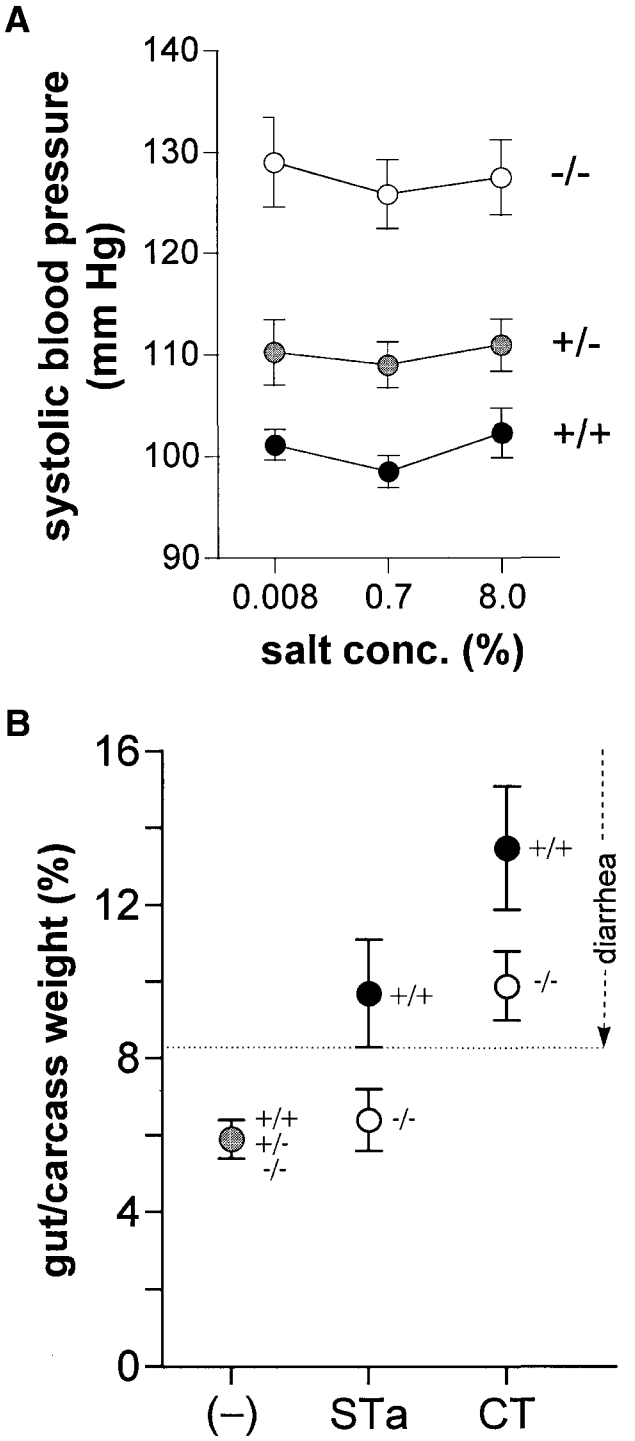

Fig. 3. A: Salt-resistant hypertension in GC-A null mice. Systolic blood pressure is significantly elevated in the heterozygous and homozygous null mice but is resistant to changes in dietary salt. B: Resistance to STa-induced diarrhea in GC-C null mice. The suckling mouse assay [31] was used to assess the response to sham, STa or cholera toxin (CT) treatment. Whereas wild-type mice display marked accumulation of fluid within the intestine in response to STa, the GC-C null mice fail to respond to the enterotoxigenic agent. Cholera toxin, however, which acts through the cAMP signalling pathway, continues to cause diarrhea in GC-C null mice. It is concluded that GC-C is the receptor that mediates the diarrhetic response to STa. Diarrhea is defined as gut/carcass weight over 0.083 (8.3%).

tern from concave upward to linear as a function of MnGTP concentration [26].

2.4. Gene disruption

Although there are many caveats to interpretations of function based on disruption of a gene, it offers a means to interrupt a signaling pathway in the intact animal and to potentially identify a corresponding phenotype in the human in addition to unraveling the functions of the signaling pathway.

2.4.1. GC-A gene disruption. GC-A deficient mice display a salt-resistant form of hypertension (Fig. 3A; [27]). The gene for ANP, an apparent ligand of GC-A, also has been disrupted, and although the initial phenotype was reported as a salt-sensitive form of hypertension [28], later studies suggested that the mice displayed a phenotype more consistent with that of the GC-A null mice [29]. The salt-resistant hypertension raised the question of whether another receptor for ANP existed within the kidney or whether the heart could release other natriuretic/diuretic factors in response to increased vascular volume. Infusion of ANP failed to cause natriuresis or diuresis in the GC-A null mice suggesting that GC-A is the exclusive ANP receptor in the kidney. Expansion of vascular volume by intravenous infusion of an isoncotic solution resulted in release of granules from the heart; however, there again was no diuresis or natriuresis, thus suggesting that GC-A is the primary receptor in the kidney for any substance that might be released from the heart to mediate natriuresis/diuresis under conditions of acute volume expansion [30], at least when volume is expanded through intravenous infusion.

2.4.2. GC-C gene disruption. GC-C is expressed principally in the intestine of the mouse. Heat stable enterotoxins (STa), which cause an acute secretory diarrhea, have been suggested to mediate their actions through the guanylyl cyclase-C (GC-C) receptor. The *GC-C* gene was disrupted by homologous recombination and homozygous null mice contained no detectable GC-C mRNA or protein [31]. Intestinal mucosa guanylyl cyclase activity was about 16-fold higher in wild-type mice than in the GC-C null mice suggesting that GC-C is the major guanylyl cyclase activity present in the intestine. It has been a point of controversy for some years whether there are multiple receptors for STa that mediate diarrhea. When ^{125}Sta binding or STa-stimulable guanylyl cyclase activity was measured, however, it was about one-half wild-type mice in heterozygous animals and absent in the GC-C null animals. Thus, GC-C completely accounts for the STa-induced elevations of cGMP. While gavage with STa results in marked fluid accumulation within the intestine of wild-type and heterozygous suckling mice, GC-C null animals are resistant to such treatment (Fig. 3B). In addition, infection with enterotoxigenic bacteria that produce STa leads to diarrhea and death in wild-type and heterozygous mice, while the null mice are protected. Cholera toxin, in contrast, continues to cause diarrhea in GC-C null mice, demonstrating that the cAMP signaling pathway remained intact. Markedly different diets (high carbohydrate, fat, or protein) or the inclusion of high salt (K^+, Na^+) in the drinking water or diet also did not severely affect the null animals. Given that GC-C is a major intestinal receptor in all mammals, the pressure to retain a functional GC-C in the face of diarrhea-inflicted mortality remains unexplained. GC-C, therefore, likely provides a necessary protective effect against stressors not yet tested, possi-

bly pathogens other than non-invasive enterotoxigenic bacteria.

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