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# Guanylyl Cyclase C (GC-C) Inhibits Human Colon Carcinoma Cell Growth

Giovanni Mario Pitari

*Thomas Jefferson University, gmpitari@gmail.com*

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# GUANYLYL CYCLASE C (GC-C) INHIBITS HUMAN COLON CARCINOMA CELL GROWTH

**Giovanni Mario Pitari**

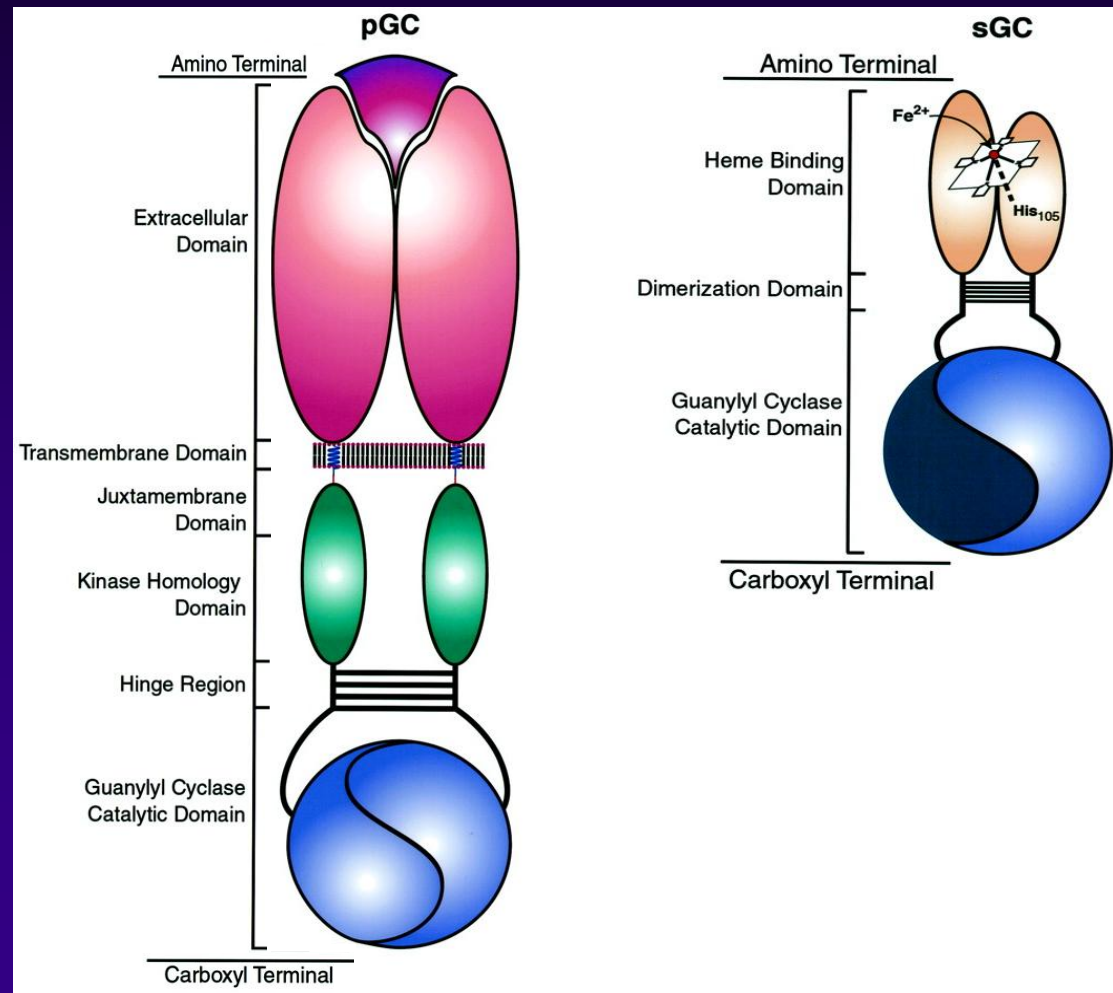
*Division of Clinical Pharmacology*

*Department of Medicine*

*Thomas Jefferson University*

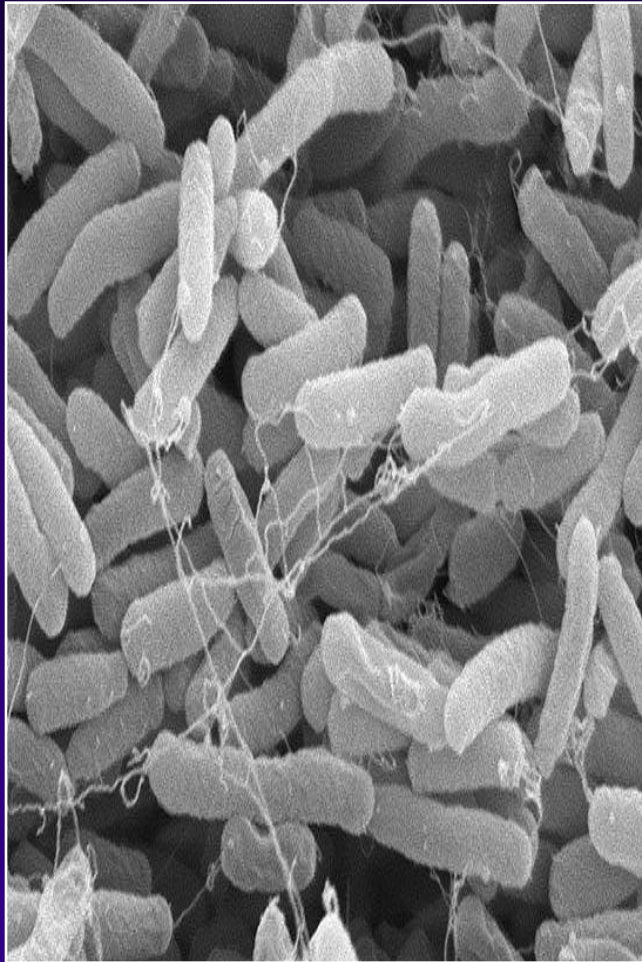
*Philadelphia, PA 19107*

# Guanylyl Cyclase Family





# The E. coli Heat-Stable Enterotoxin (ST) Binds GC-C



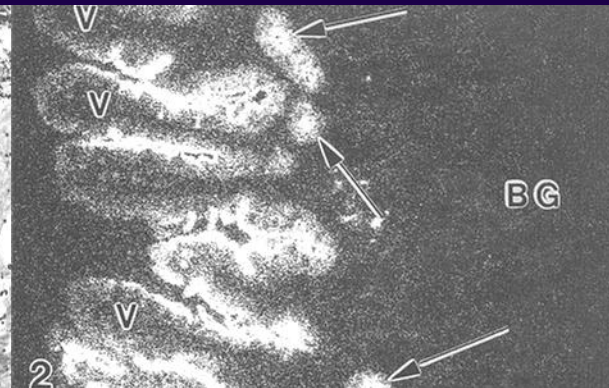
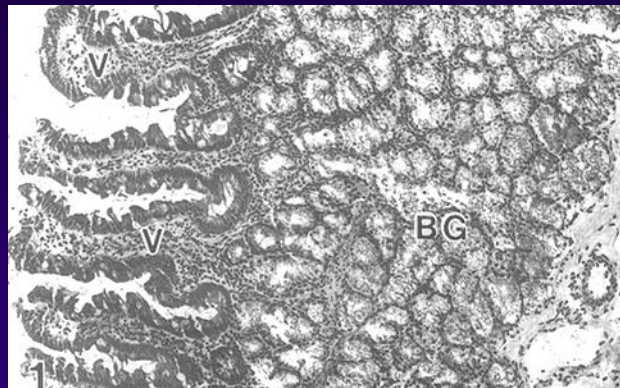
NTFYCCELCCNPACAGCY



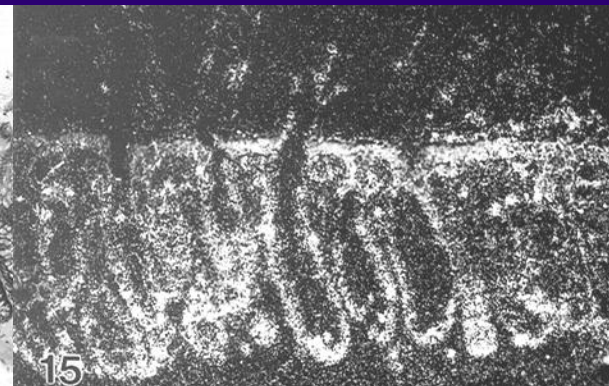
# GC-C is Localized to Intestinal Epithelial Cells

H&E

$^{125}\text{I}$ -ST Binding

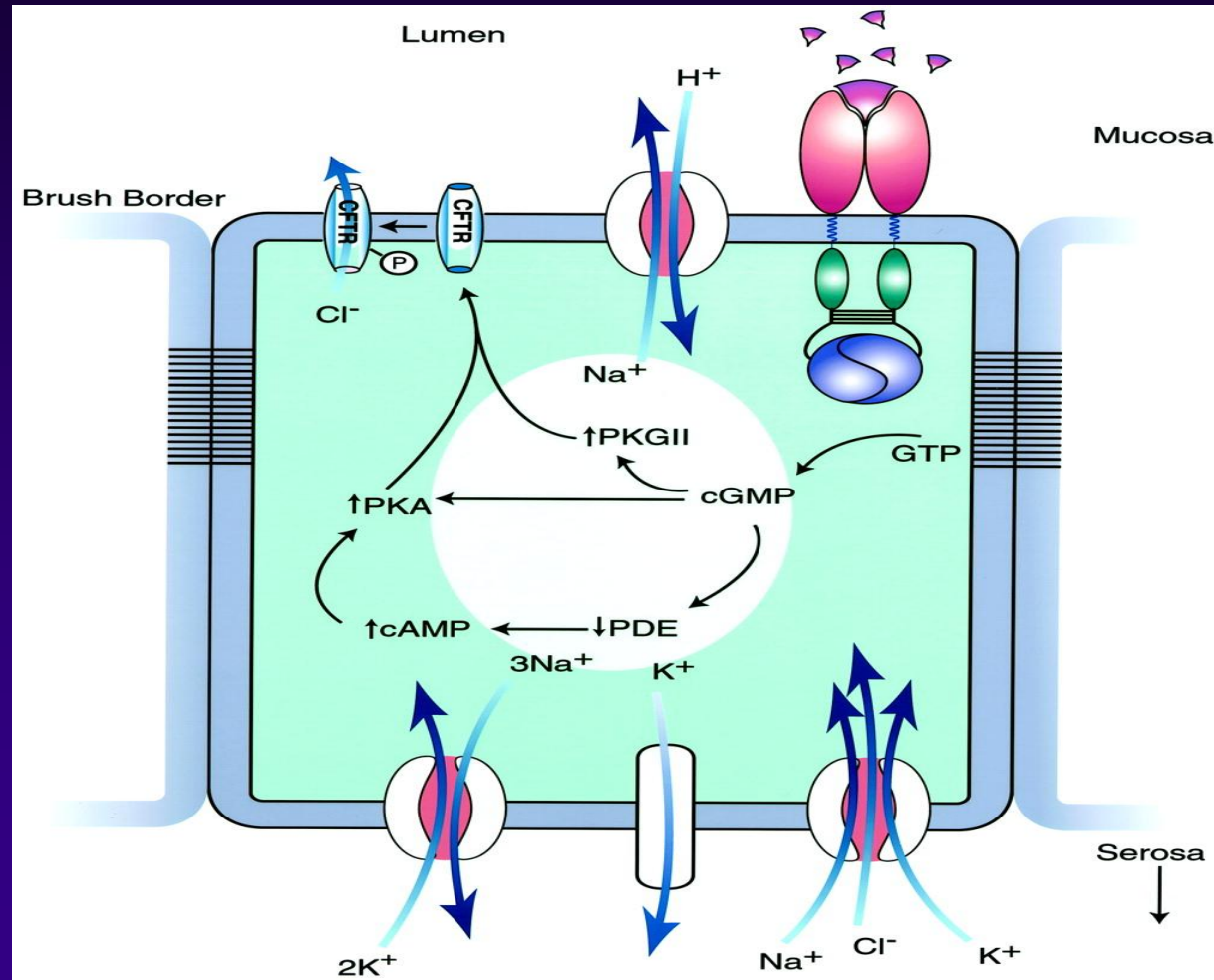


Small  
Intestine



Large  
Intestine

# GC-C Signaling Cascade





# Does GC-C Mediate More Than Fluid Transport in Intestine?

- *Does GC-C regulate intestinal epithelial cell proliferation?*
- *What are the molecular mechanisms by which GC-C regulates intestinal cell proliferation?*



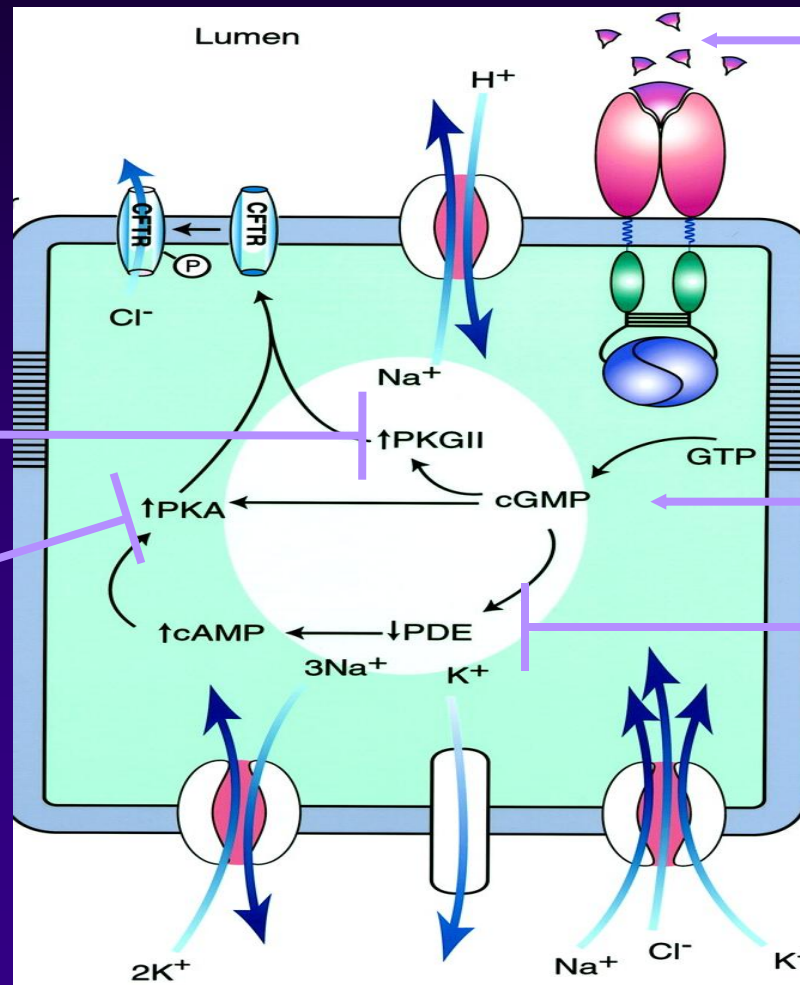
# Protocol Design & Materials

**Cell Lines:**  
T84, Caco-2, SW480

**Pro-Proliferative Agents:**  
FBS, L-Glutamine

**KT5823**  
**RP8pCPT-cGMP**

**KT5720**  
**Rp-cAMPs**



**ST**  
**Uroguanylin**

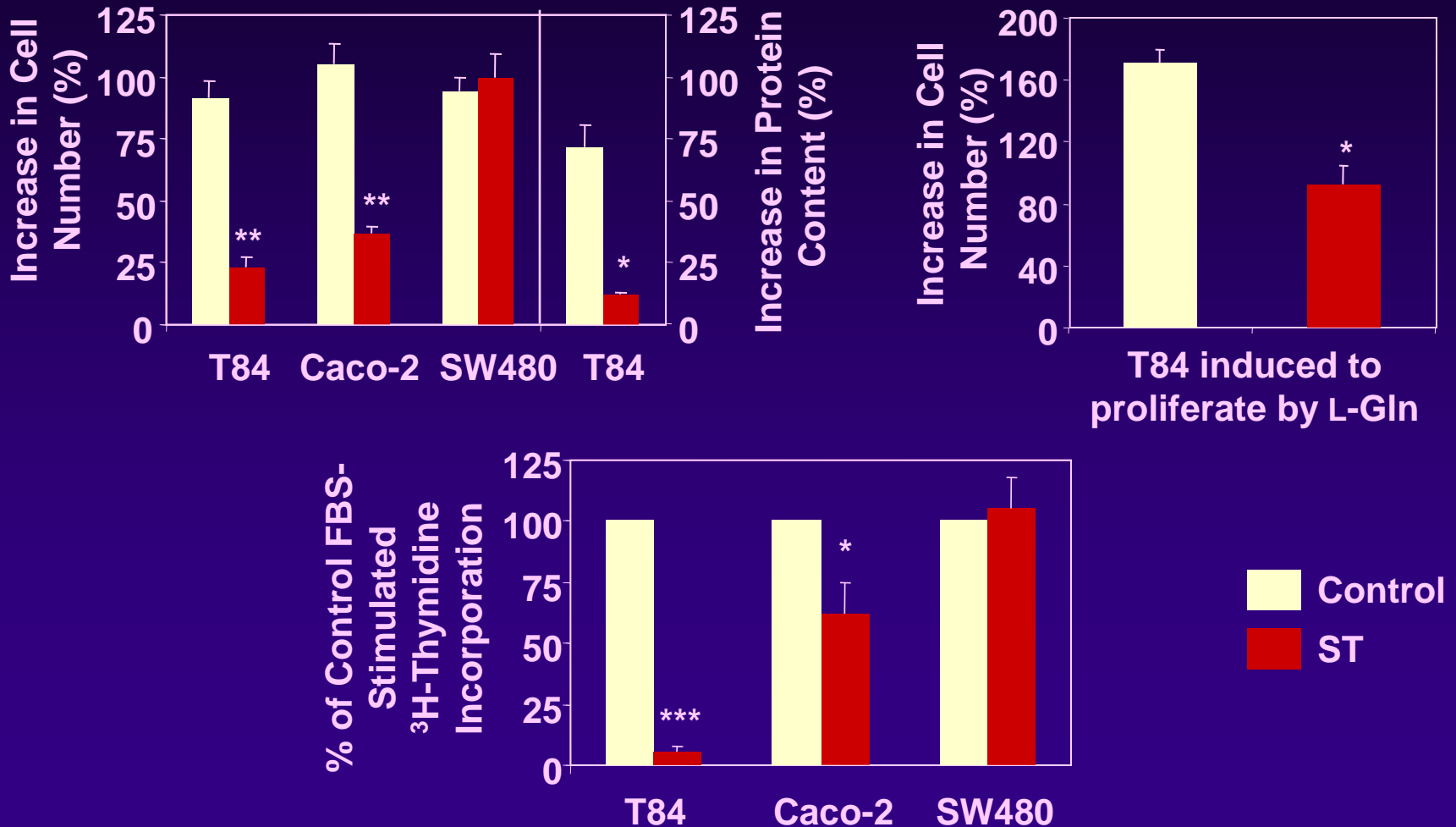
**8-Br-cGMP**

**Milrinone**



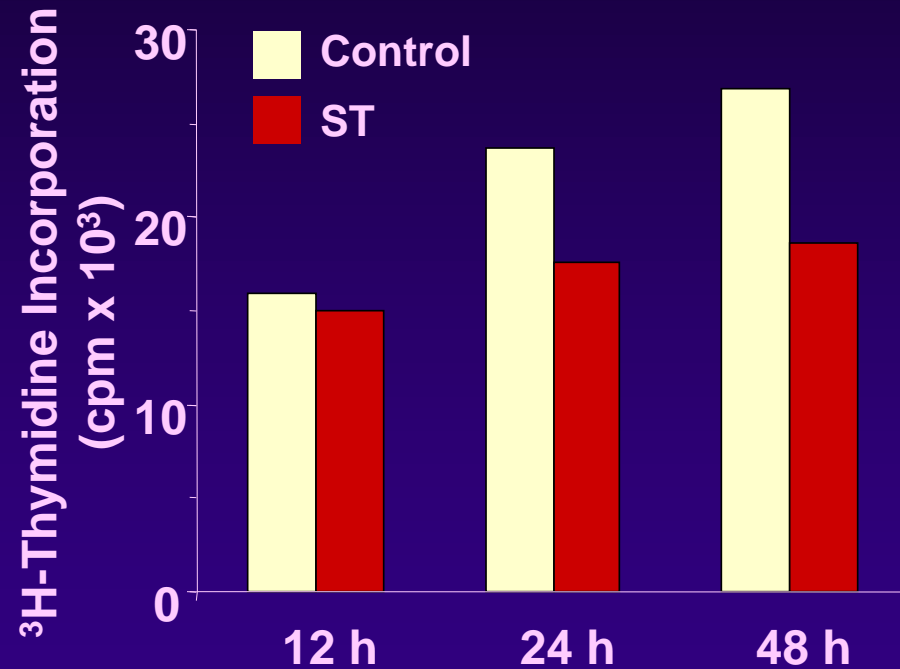
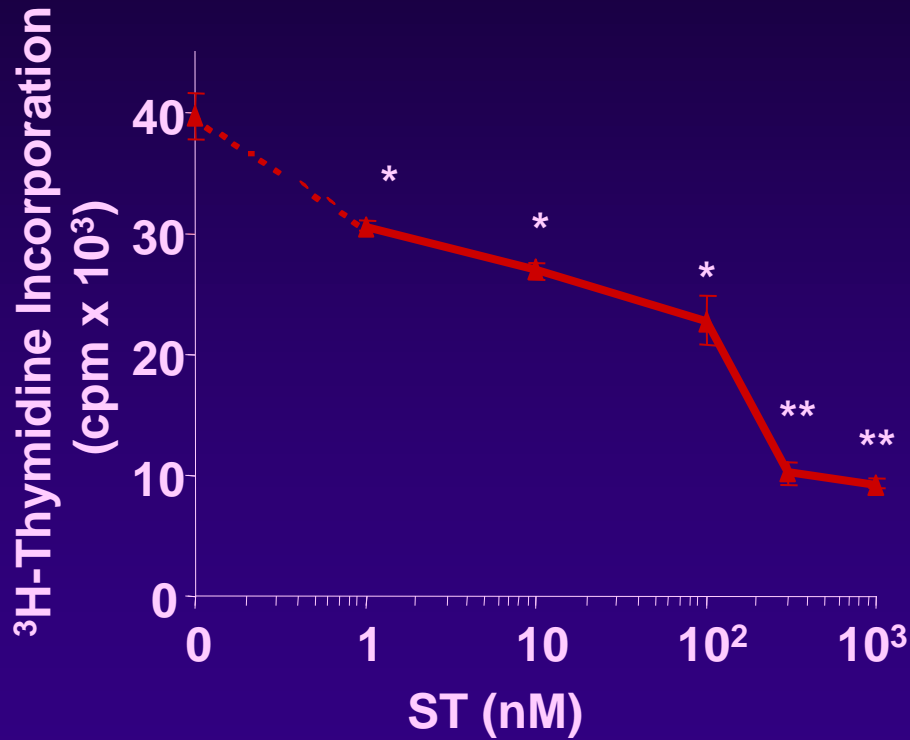


# ST Inhibits Intestinal Cell Proliferation



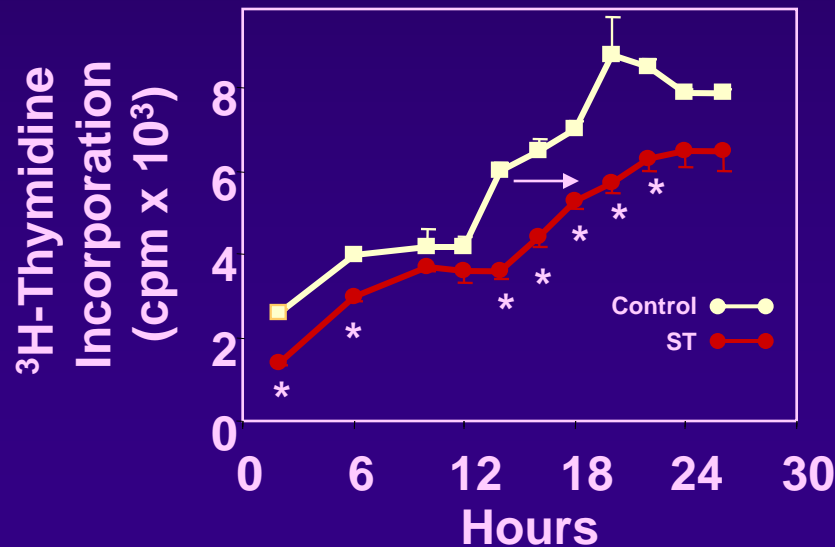
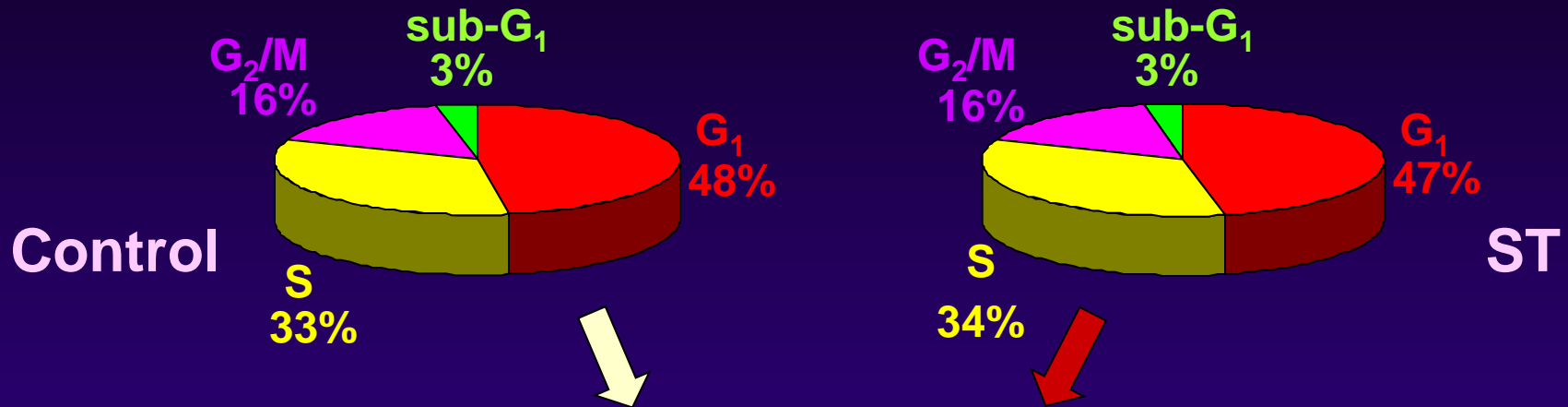


# ST Inhibition is Dose- and Time-Dependent



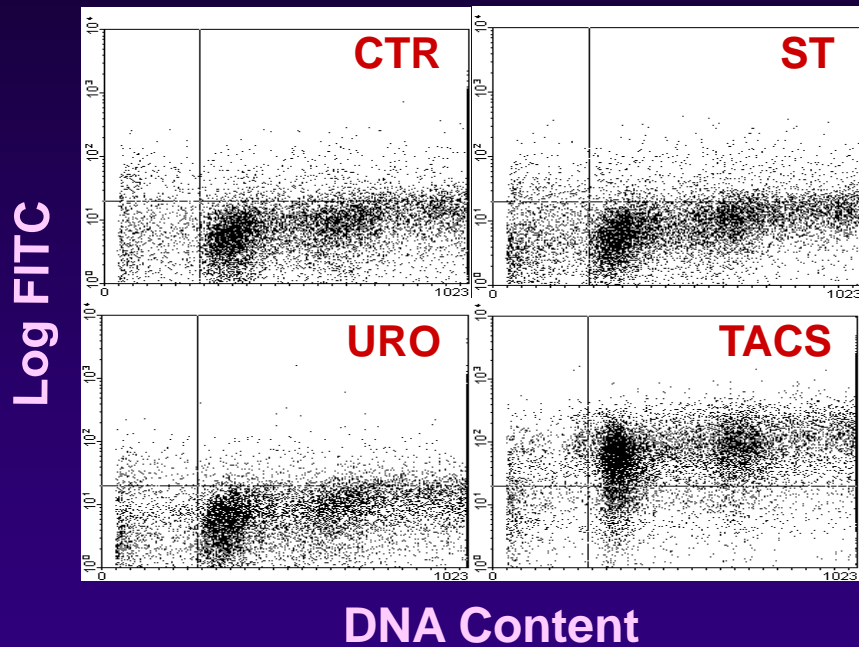


# ST Delays, But Does Not Arrest, the Cell Cycle





# GC-C Agonists Do Not Induce Apoptosis or Necrosis



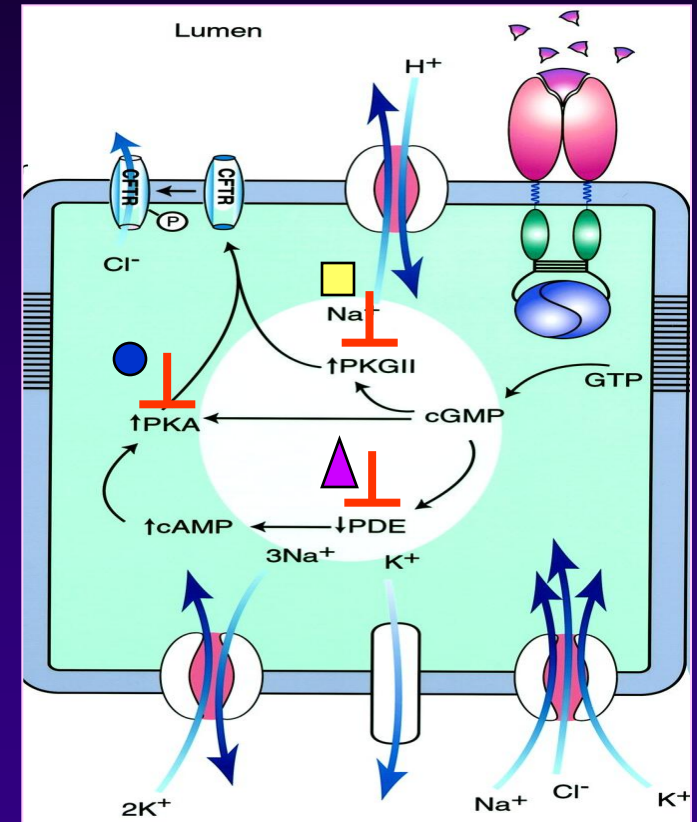
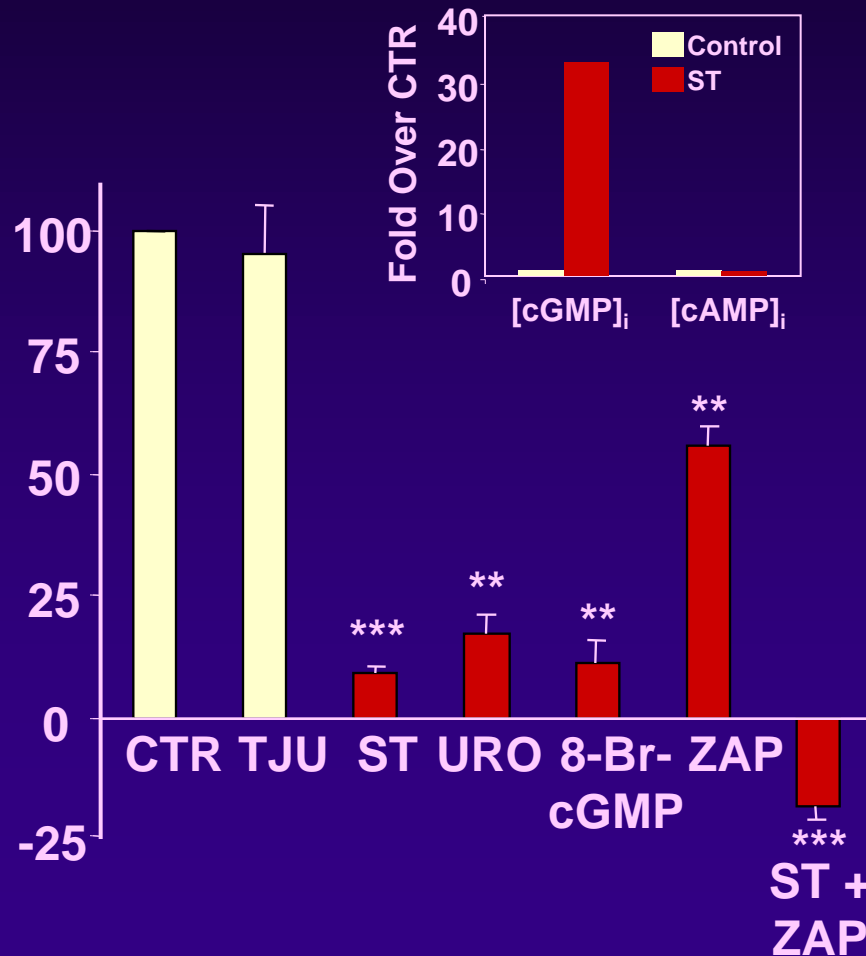
	Control	ST (1 $\mu$ M)	Uro (1 $\mu$ M)	TACS
% Apoptosis	7.4 $\pm$ 0.5	9.1 $\pm$ 1.2	6.9 $\pm$ 0.9	75.3 $\pm$ 2.1**

\*\* p<0.01



# ST Cell Signaling Pathway for the Inhibition of Proliferation

GLN-stimulated Thymidine Incorporation (%)



- ▲ Milrinone
- KT5823, RP8pCPT-cGMP
- KT5720, Rp-cAMPs



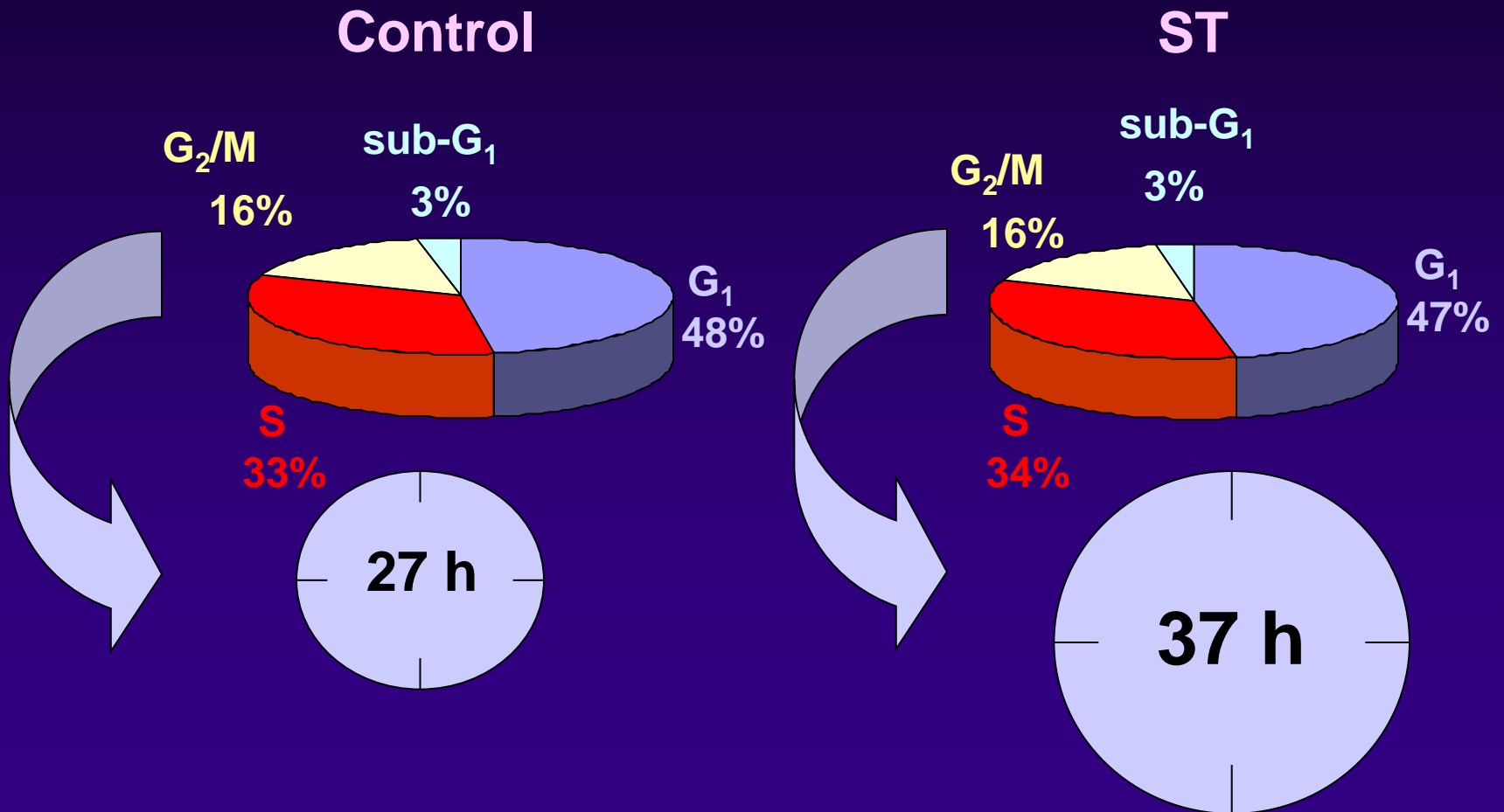
# Summary

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- *GC-C activation inhibits colon carcinoma cell proliferation in vitro*
- *Inhibition of proliferation results from a prolongation of the cell cycle, not cell death*
- *The cytostatic effect of ST is mediated by an increase in  $[cGMP]_i$*



# ST-Dependent Cytostasis Does Not Reflect Arrest, but Retardation, of the Cell Cycle





# Implications of GC-C Regulation of Proliferation

- *Endogenous GC-C ligands (guanylin and uroguanylin) may represent cell cycle regulators*
- *Along the crypt-to-villus axis, GC-C may regulate the transition of intestinal epithelial cells from proliferative to differentiated states*
- *GC-C agonists may be utilized as novel cytostatic agents for the prevention and treatment of colorectal cancer*





# Acknowledgements

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