IV. What we can actually do and/or are really doing

Two examples

IVa Metabolic networks

IVb Protein folding and aggregation

Metabolic networks

- The case of the WNT pathway

  the context and the problem

- Modelization

  data and approximations

- Results

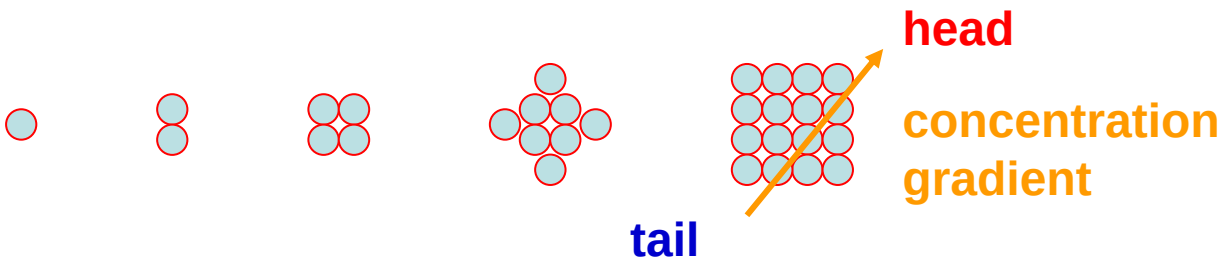
  some numerics

- Outlook

  understanding cancer onset (?)

A paradigmatic case: the WNT pathway

- Morphogenes are proteins that specify the different cell fate in a **concentration** dependent way
- WNT, Hh, BMP, … regulator proteins that (during embryogenesis) provide positional information and organize embryonic patterning



- WNT-signalling mechanism is much studied, because defects in its regulation ultimately lead to cancer
- Normally WNT regulates the level of β-catenine in the cell

1) In the absence of a WNT signal, a multi-component destruction complex, containing GSK3, Axin, ACP,… promotes Phosphorilation of β-catenine, making it ready for degradation by β-TRCP (an E3 Ubiquitin ligase)

2) In the presence of a WNT signal, the activity of the destruction complex is inhibited, and the level of cytoplasmatic β-catenine rises

β-catenine becomes complexed with the transcription factor TCF and activate TCF-target genes (c-myc, cyclinD1, tcf-1,…), which directly influence cell development processes

Accumulation of β-catenine in the cell and/or deregulation of the TCF/β-catenine activity can promote carcinogenesis in many tissues

- Mutations in the β-catenine gene CTNNb1 with consequent protein alterations (mostly in the region S29-K49)
- Defects in the WNT pathway, resulting in a deregulation of the cytoplasmatic β-catenine level

• Modeling the canonical WNT pathway

Lee Salic Krueger Heinrich Kirschner
PloS Biology, **1** (2003) 116

MAIN COMPONENTS

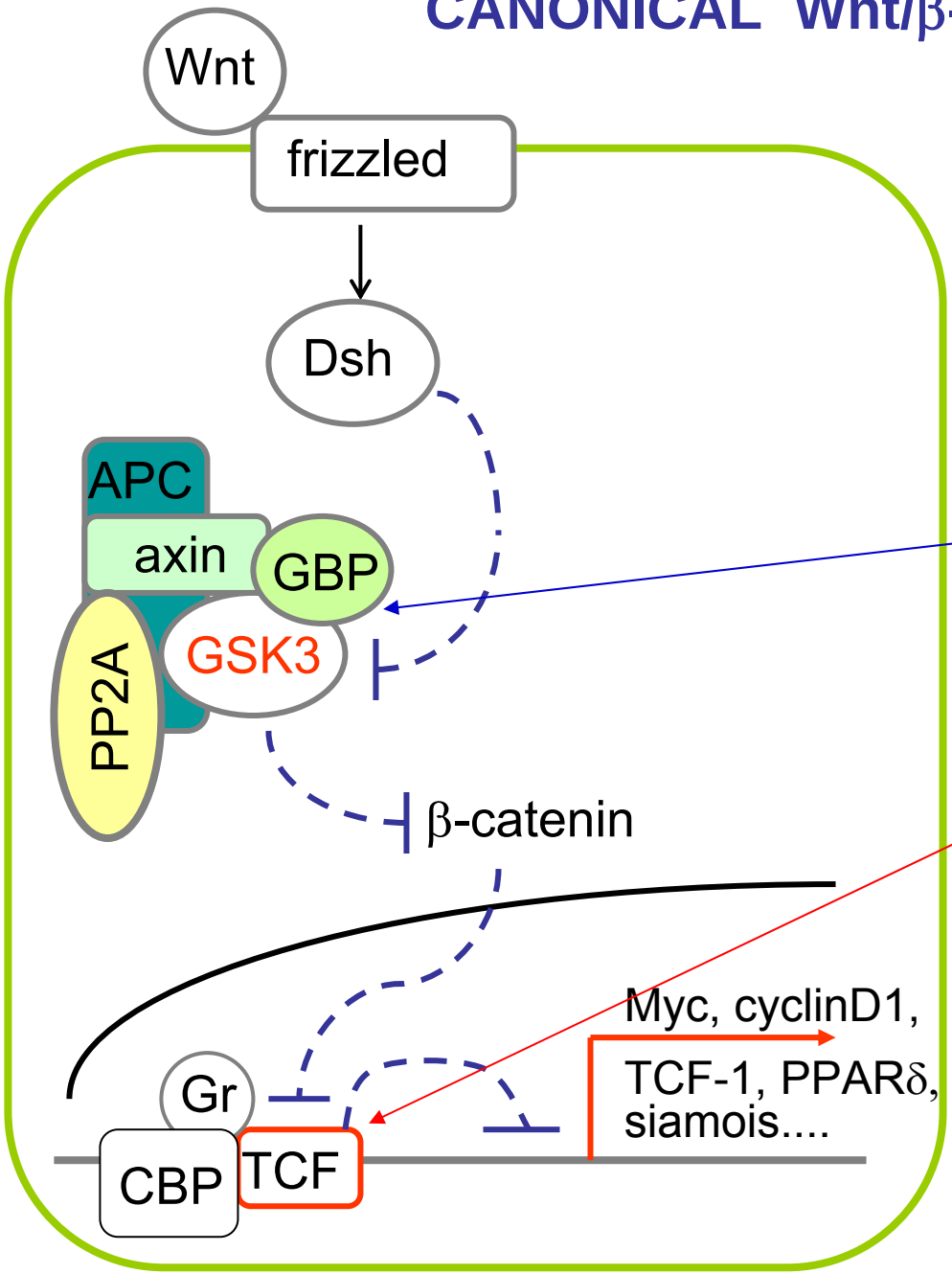
WNT (ligand)
FRIZZLED (receptor)
DISHEVELLED
AXIN (scaffold)
APC (scaffold)
GSK3 (Kinase)
GBP (**GSK3** binding protein)
PHOSPHATASE (**PP2A**)
CASEINE KINASE
β-CATENINE (transcription coactivator)
TCF (transcription factor)

and many, many more …

MUTATIONS IN APC PLAY A PARTICULARLY IMPORTANT ROLE IN COLORECTAL CANCER

APC: ADENOMATOUS POLYPOSIS COLIPROTEIN

CANONICAL Wnt/β-CATENINE PATHWAY



LOGIC OF THE PATHWAY

Either the destruction complex forms

or β-catenin gets complexed with TCF

WITH ACTIVATION OF

WNT-TARGET GENES

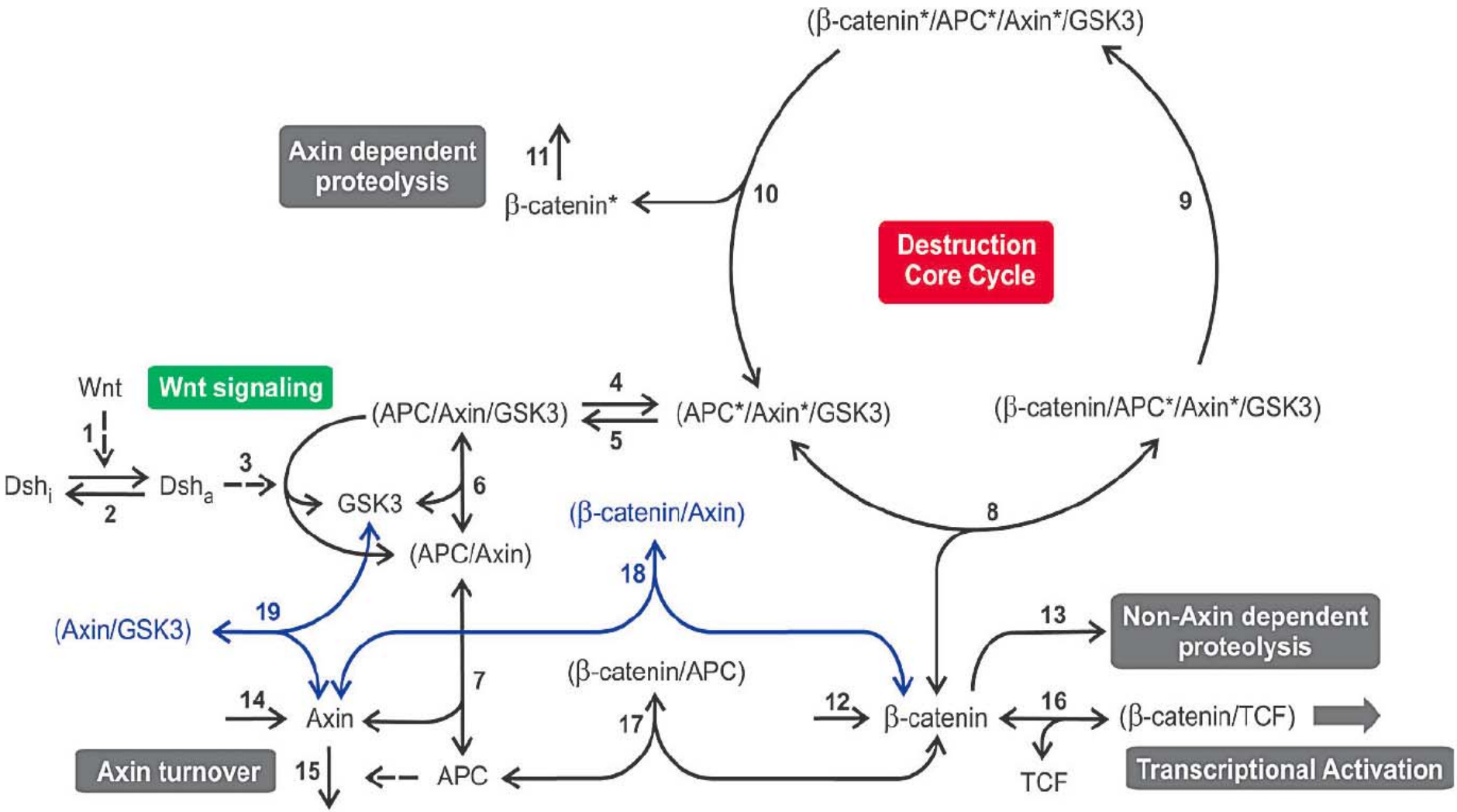
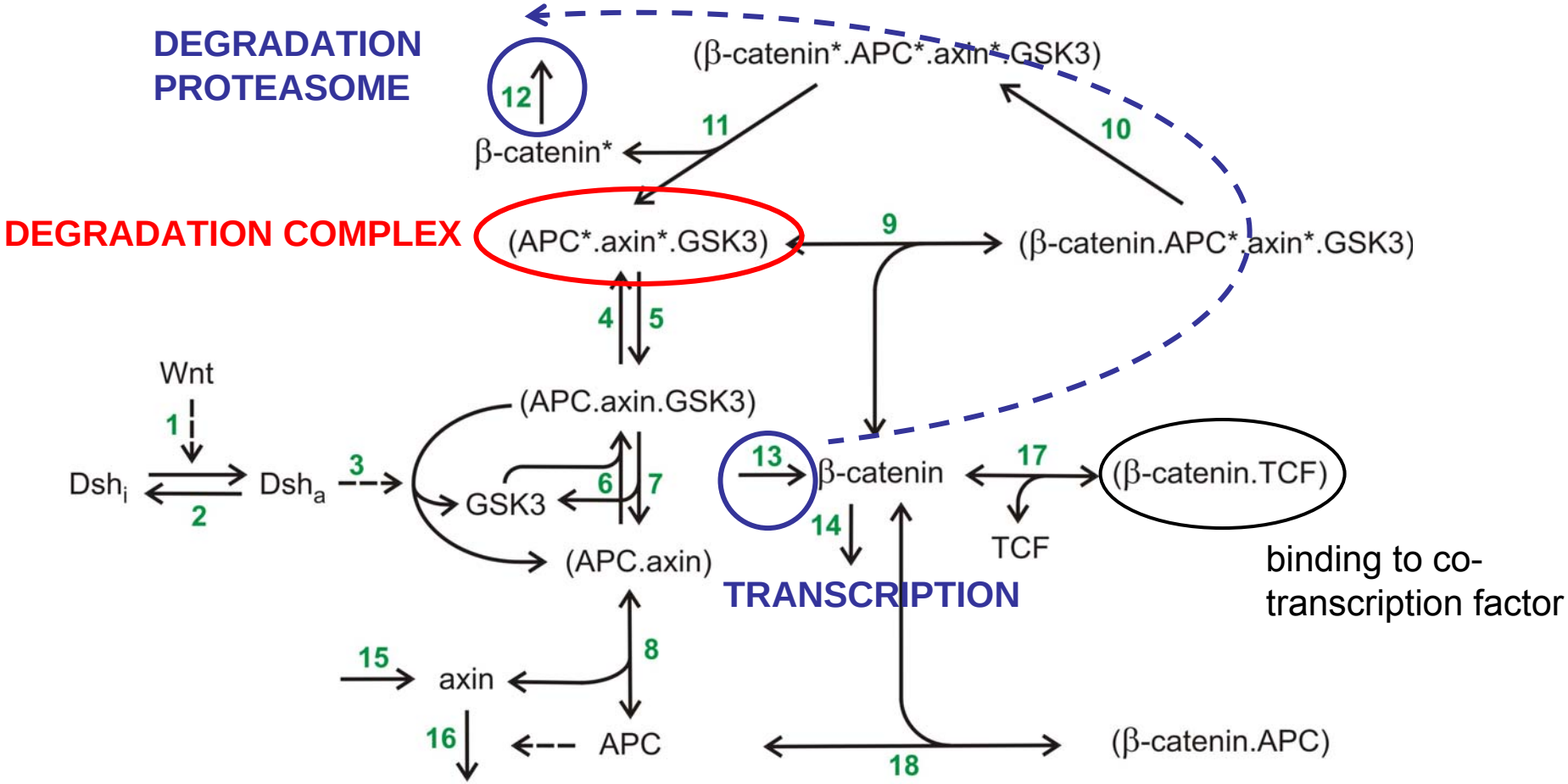


Figure 1. Reaction Scheme for Wnt Signaling

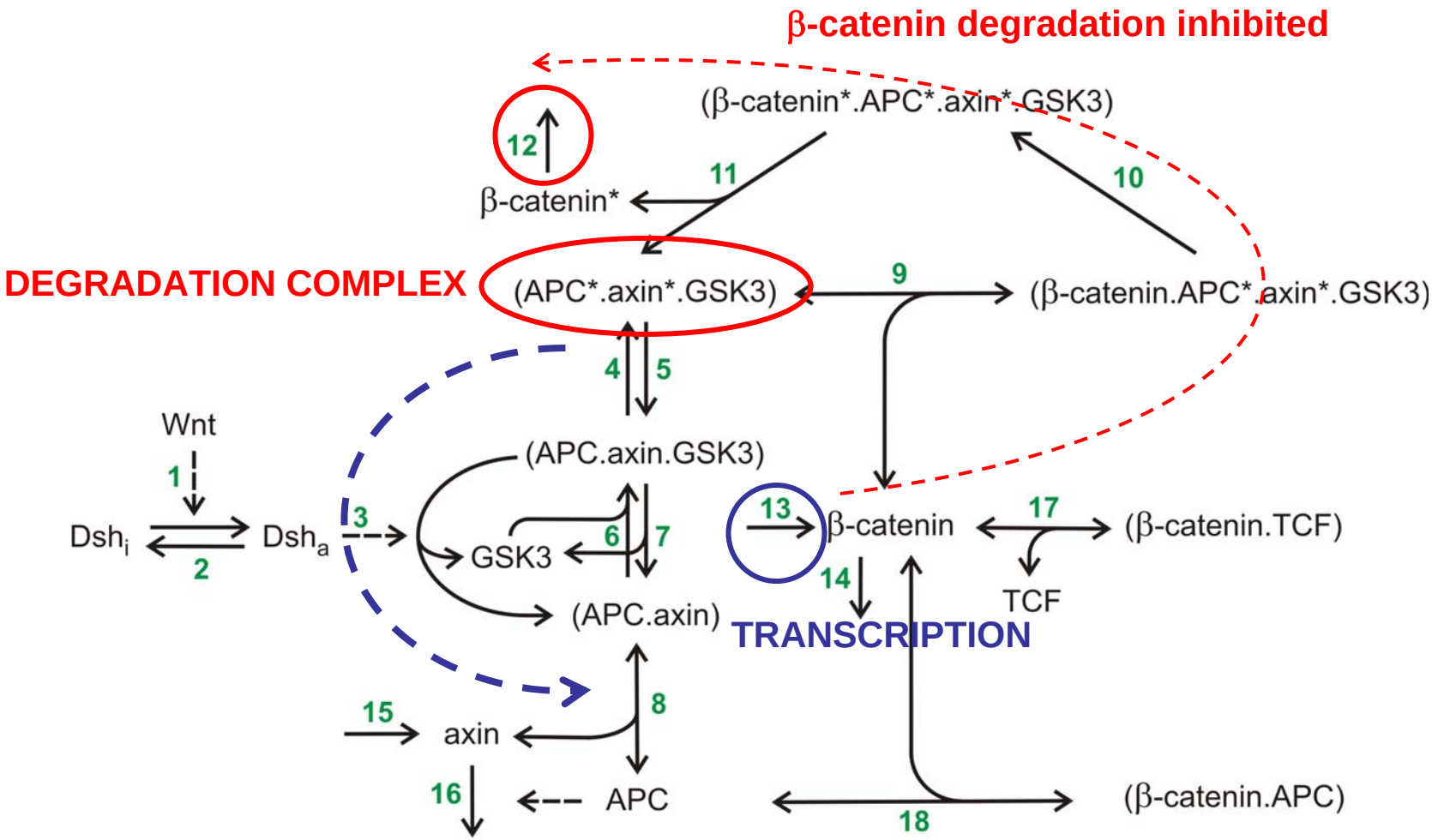
The reaction steps of the Wnt pathway are numbered 1 to 19. Protein complexes are denoted by the names of their components, separated by a slash and enclosed in brackets. Phosphorylated components are marked by an asterisk. Single-headed solid arrows characterize reactions taking place only in the indicated direction. Double-headed arrows denote binding equilibria. Blue arrows mark reactions that have only been taken into account when studying the effect of high axin concentrations. Broken arrows represent activation of Dsh by the Wnt ligand (step 1), Dsh-mediated initiation of the release of GSK3β from the destruction complex (step 3), and APC-mediated degradation of axin (step 15). The broken arrows indicate that the components mediate but do not participate stoichiometrically in the reaction scheme. The irreversible reactions 2, 4, 5, 9–11, and 13 are unimolecular, and reactions 6, 7, 8, 16, and 17 are reversible binding steps. The individual reactions and their role in the Wnt pathway are explained in the text.

DOI: 10.1371/journal.pbio.0000010.g001

Unstimulated reference state
Absence of Wnt



Effect of Wnt-stimulation



ACCUMULATION OF β-CATENIN

MAIN INPUT DATA OF THE MODEL

CONCENTRATIONS

total Dsh	**100 nM**
total APC	**100 nM**
total TCF	**15 nM**
total GSK3	**50 nM**
total axin	**0.02 nM**
total β-catenin	**35 nM**
free phosphorylated β-catenin	*1 nM*

DISSOCIATION CONSTANTS

binding of GSK3 to (APC.axin)	*10 nM*
binding of APC to axin	*50 nM*
binding of β-catenin to (APC.axin.GSK)	*120 nM*
binding of β-catenin to TCF	*30 nM*
binding of β-catenin to APC	*1200 nM*

FLUXES

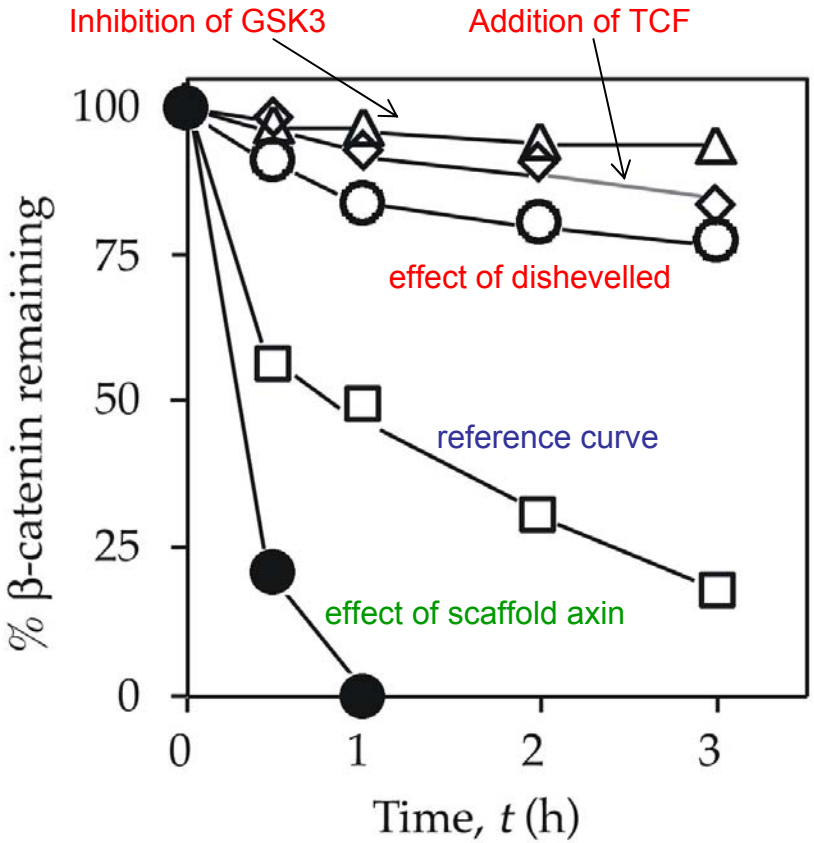
degradation flux of β-catenin via the proteasome	**25 nM/h**
Share of degradation of β-catenin via unphosphorylated form	**1.5 %**

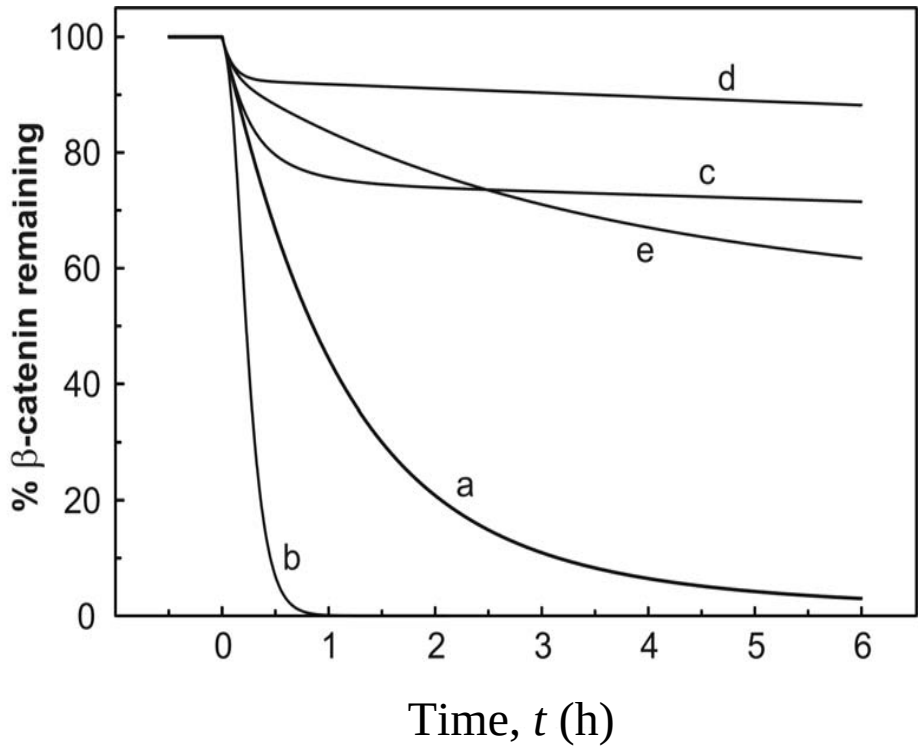
CHARACTERISTIC TIMES

phosphorylation/dephosphorylation of APC and axin	*2.5 min*
GSK3 association/dissociation	*1 min*
Axin degradation	**6 min**

• Results

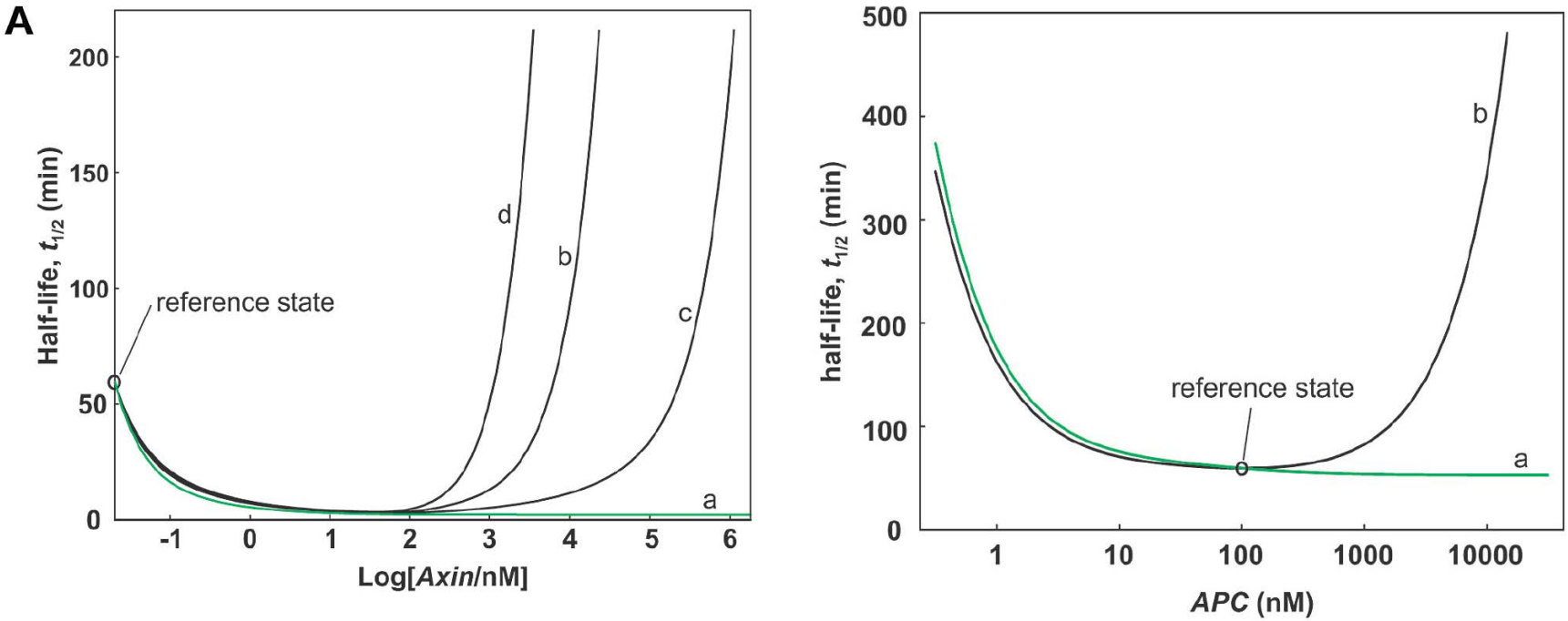
β-catenin degradation,
simulations and comparison with experimental data





• Outlook

Tumor suppressor role of Axin and/or APC?



Very complicated to devise a winning strategy (non-linear dynamics)

- Axin degradation is APC dependent
- Axin and APC both involved in the β-catenin destruction complex

HUMBOLDT-UNIVERSITY

Reinhart Heinrich

SIGNAL TRANSDUCTION

Thomas Höfer

Roland Krüger

Holger Nathansen

METABOLIC NETWORKS

Oliver Ebenhöh

Edda Klipp

Stefan Schuster

Jana Wolf

HARVARD MEDICAL SCHOOL, BOSTON

Marc Kirschner

Leon Murphy

Benjamin G. Neel

Tom A. Rapoport

Adrian Salic

Ethan Lee

Stefanie Schalm

UNIVERSITY BORDEAUX II

Jean-Pierre Mazat

Christine Reder

BIOCENTRUM AMSTERDAM

Hans Westerhoff

Roel van Driel

Martijn Moné

Summary

what can be/was done about metabolic networks

- Bio-chemical data suggest the set of relevant
  - compounds/reaction constants
  - chemical reactions
  - network topology
- Construct the set of (non-linear) diff. eqs (time and space) for concentrations
- Solution
  - identify relevant initial states
  - evolve the equations
  - stability studies around different points in concentration space
- Devise experiments and compare
- Identify the key features of the system
  - compounds/reaction constants
  - chemical reactions
  - network topology
- hence what is needed to correct what goes wrong

(like accumulation of β-catenin in adult cell, as it would promote unwanted expression of the silenced TRCβ gene)