Biochemistry of Cancer

Tumor Suppressor Genes

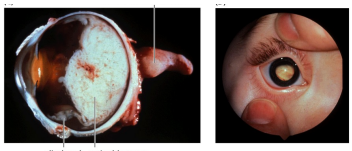


Figure 7.3 The Biology of Cancer (© Garland Science 2014)

Table 7.1 Examples of human tumor suppressor genes that have been cloned

Name of gene	Chromosomal location	Familial cancer syndrome	Sporadic cancer	Function of gene product
SDHB	1p36.1	paraganglioma	—	succinate dehydrogenase
CHD5	1p36.31	cutaneous melanoma	many types	histone reader, transcriptional inducer
HRPT2	1q25–32	parathyroid tumors, jaw fibromas	parathyroid tumors	chromatin protein
FH	1q42.3	familial leiomyomatosis[a]	—	fumarate hydratase
FHIT	3p14.2	—	many types	diadenosine triphosphate hydrolase
BAP1	3p21.1	mesothelioma, melanoma	mesothelioma, uveal melanoma	ubiquitin hydrolase
RASSF1A	3p21.3	—	many types	multiple functions
TGFBR2	3p2.2	HNPCC	colon, gastric, pancreatic carcinomas	TGF-β receptor
VHL	3p25–26	von Hippel–Lindau syndrome	renal cell carcinoma	ubiquitylation of HIF
hCDC4	4q32	—	endometrial carcinoma	ubiquitin ligase
APC	5q21–22	familial adenomatous polyposis coli	colorectal, pancreatic, and stomach carcinomas; prostate carcinoma	β-catenin degradation
NKX3.1	8p21.2	—	prostate carcinoma	homeobox TF
miR-124a[b]	8p23.1	—	many types	suppresses CDK6
$p16^{INK4A}$ [c]	9p21	familial melanoma	many types	CDK inhibitor
$p14^{ARF}$ [d]	9p21	—	all types	p53 stabilizer

Table 7.1 Examples of human tumor suppressor genes that have been cloned

Name of gene	Chromosomal location	Familial cancer syndrome	Sporadic cancer	Function of gene product
PTC	9q22.3	nevoid basal cell carcinoma syndrome	medulloblastomas	receptor for hedgehog GF
let 7a (miRNA)[e]	9q22.32	—	many types	suppresses Ras, Myc
TSC1	9q34	tuberous sclerosis	—	inhibitor of mTOR[f]
BMPR1	10q21–22	juvenile polyposis	—	BMP receptor
ANXA7	10q21	—	breast, prostate, stomach	endocytosis
PTEN[g]	10q23.3	Cowden's disease, breast and gastrointestinal carcinomas	glioblastoma; prostate, breast, and thyroid carcinomas	PIP_3 phosphatase
WT1	11p13.5–6	Wilms tumor	Wilms tumor	TF
MEN1	11p13	multiple endocrine neoplasia	—	histone modification, transcriptional repressor
BWS/CDKN1C	11p15.5	Beckwith–Wiedemann syndrome	—	$p57^{Kip2}$ CDK inhibitor
SDHD[h]	11q23.1	paraganglioma, pheochromocytoma	pheochromocytoma	mitochondrial protein
CBL	11q23.3	juvenile myelomonocytic leukemia	adult myelomonocytic leukemia	SH2-containing ubiquitin ligase
RB	13q14.2	retinoblastoma, osteosarcoma	retinoblastoma; sarcomas; bladder, breast, esophageal, and lung carcinomas	transcriptional repression; control of E2Fs
miR-15a/16-1	13q14.3	—	B-cell lymphoma	suppresses Bcl-2, Mcl-1, cyclin D1, Wnt3a

Table 7.1 Examples of human tumor suppressor genes that have been cloned

Name of gene	Chromosomal location	Familial cancer syndrome	Sporadic cancer	Function of gene product
RUNX1	21q22.12	familial platelet disorder	AML	TF
SNF5[i]	22q11.2	rhabdoid predisposition syndrome	malignant rhabdoid tumors	chromosome remodeling
NF2	22q12.2	neurofibroma-predisposition syndrome	schwannoma, meningioma; ependymoma	cytoskeleton–membrane linkage
WTX	Xq11.1	—	Wilms tumor	β-catenin degradation

[a]Familial leiomyomatosis includes multiple fibroids, cutaneous leiomyomas, and renal cell carcinoma. The gene product is a component of the tricarboxylic cycle.
[b]*miR124a-1* genes are also located at 8q12.3 and 20q13.33l.
[c]Also known as *MTS1*, *CDKN2*, and *p16*.
[d]The human homolog of the murine $p19^{ARF}$ gene.
[e]There are altogether 11 loci encoding let7 miRNAs in the human genome.
[f]mTOR is a serine/threonine kinase that controls, among other processes, the rate of translation and activation of Akt/PKB, TSC1 (hamartin), and TSC2 (tuberin), thereby controlling both cell size and cell proliferation.
[g]Also called *MMAC* or *TEP1*.
[h]*SDHD* encodes subunit D of the succinate dehydrogenase (succinate–ubiquinone oxidoreductase) enzyme, a component of the mitochondrial respiratory chain complex II.
[i]The *CBP* gene is involved in chromosomal translocations associated with AML. These translocations may reveal a role of a segment of CBP as an oncogene rather than a tumor suppressor gene.
[j]Also termed Carney complex.
[k]Encodes the Smad4 TF associated with TGF-β signaling; also known as *MADH4* and *SMAD4*.
[l]The human SNF5 protein is a component of the large SwiSnf complex that is responsible for remodeling chromatin in a way that leads to transcriptional repression through the actions of histone deacetylases. The rhabdoid predisposition syndrome involves susceptibility to atypical teratoid/rhabdoid tumors, choroid plexus carcinomas, medulloblastomas, and extra-renal rhabdoid tumors.

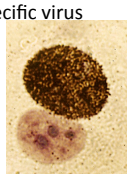
Adapted in part from E.R. Fearon, *Science* 278:1043–1050, 1997; and in part from D.J. Marsh and R.T. Zori, *Cancer Lett.* 181:125–164, 2002.

Table 7.1 (part 4 of 4) The Biology of Cancer (© Garland Science 2014)

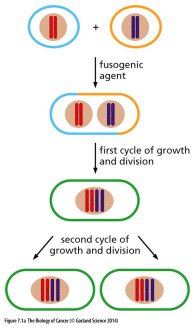
Tumor Suppressor Genes

- Recessive phenotype
  - Oncogenes (viral or cellular) dominate a phenotype – i.e. activation of cancer like behaviors. Ras, Myc, Erb – all stimulate aggressive growth, motility, invasion, migration and tumor growth
  - These are dominant cancer phenotypes
  - First hint of a different sort of cancer gene came with fusogenic experiments using PEG or specific virus

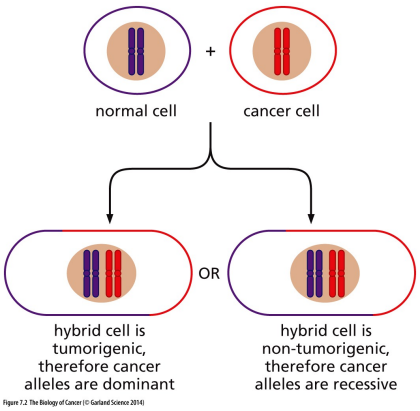
- NIH 3T3 and monkey kidney cells fused with proteins expressed by Sendai virus



Fusion Expt



- Hybrid cells – heterokaryons eventually only one set (mixed) of chromosomes will remain
- Tumor cells (expected dominant allele) did was recessive
- Indicating another gene/ allele was involved. Loss of which was only way to detect. Thus recessive.. Sort of…

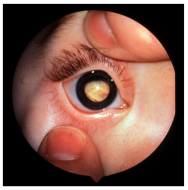


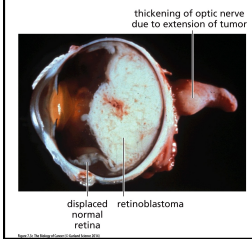
Simple Definition

- Tumor suppressor gene (aka anti-oncogene) stops cell growth protecting cell from cancer like behavior
  - Act in recessive behavior giving rise to the "two hit" hypothesis. Loss of both genes must take place before phenomenon is observed.
  - Retinoblastoma is a classic example of tumor suppressor genes
  - Some tumor suppressor genes are not recessive but "dominant negative" Mutation of one gene copy will prevent normal function. Typically a dimer were mutation allows dimer to form with wild-type protein but block its effect– p53 is an example

Retinoblasoma

- Tumor arising in youth very rare – 1 in 20,000. Occurs early. Tumor within the eye causing blindness.
- Most treatment by radiation or removal of the eye





- Two forms.
  - Those with no family history (unilateral) have tumors in one eye and is considered sporadic. No further risk of other tumors later
  - Familial form (bilateral) often have tumors in both eyes and have greater risk of other tumors forming at distal sites

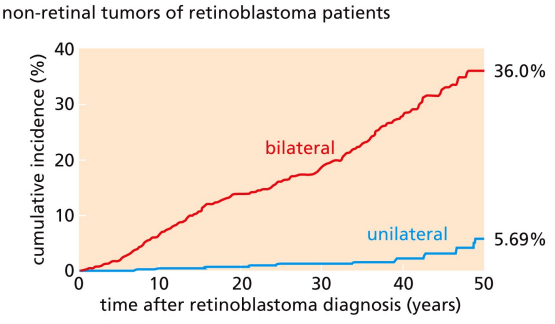


Figure 7.4a The Biology of Cancer (© Garland Science 2014)

Kinetics of age and frequency

- Suggest unilateral result from a single event while sporatic tumors require two random mutations/ events.
- Lead to speculation of an unknown gene called Rb.

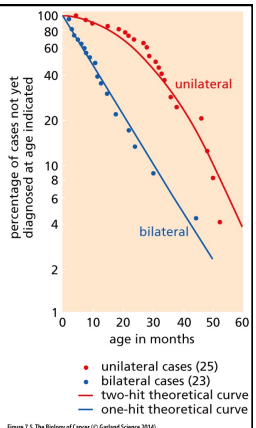
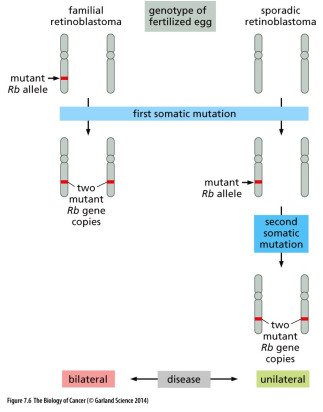


Figure 7.5 The Biology of Cancer (© Garland Science 2014)



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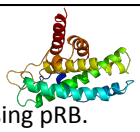
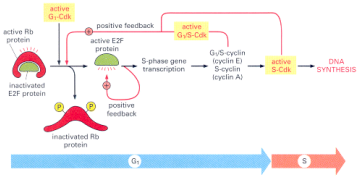
Rb function



- Retinoblastoma – RB1 gene expressing pRB.
- Phosphorlyated transcription REPRESSOR that blocks Go-G1 transition.
- Binds and blocks E2F TF which drives DNA replication. E2F is target of several oncogenes



Loss of Heterozygosity (LOH)

- Several tumor suppressors display LOH
- Removal of remaining TS gene by recombination events during mitosis
- Small genetic population interbreeding (endogamy) leads to LOH

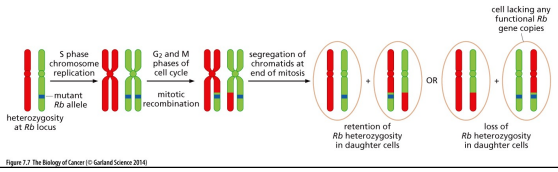
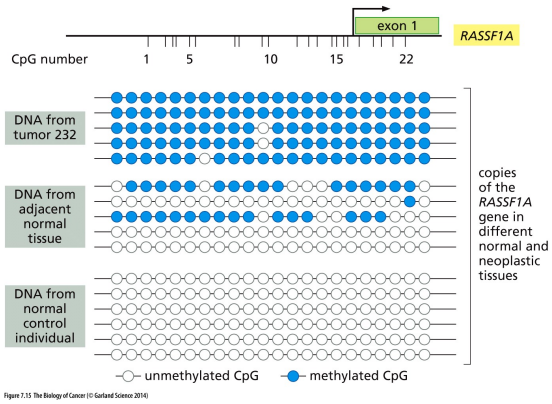


Figure 7.7 The Biology of Cancer (© Garland Science 2014)

Other mechanisms of TS loss

- Hypermethylation of cytosine bases.
- CpG (C is methylated following a G base) such CpG islands occur in some **promoter regions**
- CH_3-cytosine represses TF and Promoter binding but is reversible.
- Mechanism (not the enzyme, but control of methylation) is largely unclear at this time.
- 70% of genes have CpG islands.



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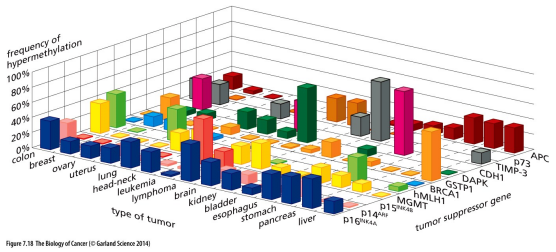


Figure 7.18 The Biology of Cancer (© Garland Science 2014)

- Example of multiple TS genes with hypermethylation in some cancers.
- Not all TS are methylated
- Height of each bar indicates the proportion of tumors of a type of tumor with hypermethylation

Table 7.2 Examples of hypermethylated genes found in human tumor cell genomes

Name of gene	Nature of protein function	Type of tumor
RARβ2	nuclear receptor for differentiation	breast, lung
p57Kip2	CDK inhibitor	gastric, pancreatic, hepatic; AML
TIMP3	inhibitor of metalloproteinases	diverse tumors
IGFBP	sequesters IGF-1 factor	diverse tumors
CDKN2A/p16INK4A	inhibitor of CDK4/6	diverse tumors
CDKN2B/p15INK4B	inhibitor of CDK4/6	diverse tumors
p14ARF	inhibitor of HDM2/MDM2	colon, lymphoma
APC	inducer of β-catenin degradation	colon carcinomas
ER	estrogen receptor	breast
p73	aids p53 to trigger apoptosis	diverse tumors
GSTP1	mutagen inactivator	breast, liver, prostate
MGMT	DNA repair enzyme	colorectal
CDH1	cell–cell adhesion receptor	bladder, breast, colon, gastric
DKK1	Wnt inhibitor	colon
DAPK	kinase involved in cell death	bladder
MLH1	DNA mismatch repair enzyme	colon, endometrial, gastric
PTEN	degrades PIP_3	diverse tumors
TGFBR2	TGF-β receptor	colon, gastric, small-cell lung
THBS1	angiogenesis inhibitor	colon, glioblastoma
VHL	ubiquitin ligase	kidney, hemangioblastoma
RB	cell cycle regulator	retinoblastoma
CASP8	apoptotic caspase	neuroblastoma, SCLC
APAF1	pro-apoptotic cascade	melanoma
CTMP	inhibitor of Akt/PKB	glioblastoma multiforme

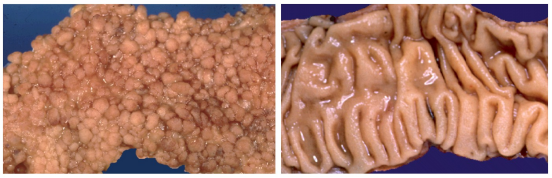
Adapted in part from C. A. Eads et al., *Cancer Res.* 61:3410–3418, 2001; and M. Esteller, *Nat. Rev. Cancer* 8:286–298, 2007.

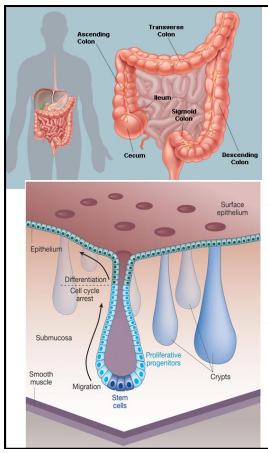
TS Genes APC – Colon Cancer

- Colon cancers – longest part of the intestine. Most are secreting mucus cell cancers (adenocarcinomas)
- Often combined with rectal cancer (last several inches of intestine) for colorectal cancer
- Most CR cancers show no or little familial association ~5% are familial adenomatous polyposis (FAP)

APC Familial Adenomatous Polyposis

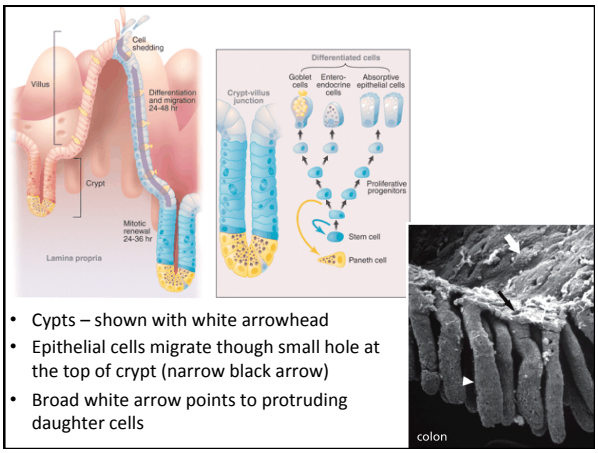
- Those with family history with parent or sibling with disease has nearly a three fold greater risk of colorectal cancer. Less than 1% of CR Cancer have FAP
- FAP patients display a carpet of polyps





Your Colon

- Colonic crypt cells provide new cells as stem cells at the bottom of the deep "cave" divide – retaining one daughter stem cell and a second daughter cell that is differentiated
- Differentiated cells migrates to luminal surface (epithelial cells) of the colon to secrete mucus and serve as the lining of the gut
- Most cells die within 3-4 days.
- Cells at surface face harsh environment of mutagenic compounds from diet, radicals from oxidation and other harsh typical conditions of intestine (pH ect…)
- Mutagens occur often in these cells but quickly die before progressing to cancer cells – thus only cancers that can happen will stop the out-migration of epithelial cells where additional lesions can induce proliferation and tumor formation can take place… APC



- Cypts – shown with white arrowhead
- Epithelial cells migrate though small hole at the top of crypt (narrow black arrow)
- Broad white arrow points to protruding daughter cells

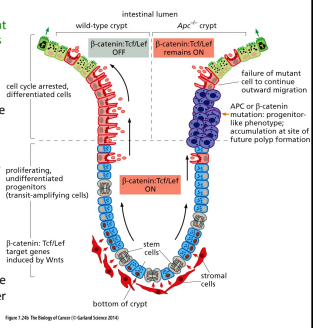
Wnt, catenin, APC & cancer

Wild-type

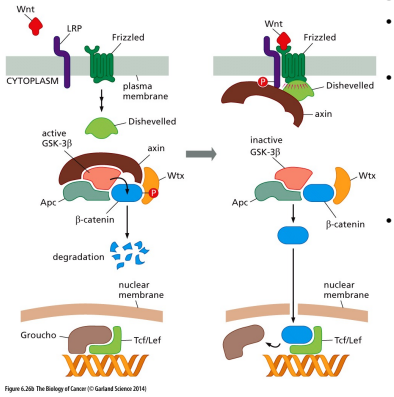
- Stromal cells (fibroblasts, inflammatory cells and endothelial cells) secrete growth factors, cytokines and other agonists including Wnt, to induce cell growth.
- The paracrine stromal cell signaling activates Wnt signaling in dividing stem and differentiated cells
- Wnt increases ß-catenin and decreases APC protein
- ß-catenin binds TF Tcf/Lef leading to increased proliferation and decreased differentiation (more stem cell like)
- APC – causes the degradation of ß-catenin
- Luminal cells are not stimulated by stromal cells, less Wnt, -> less ß-catenin and more APC (which also decreases ß-catenin). Leading to loss of proliferation (cell cycle) and more differentiated (less stem cell/cancer like) behavior and cell death

Cancer

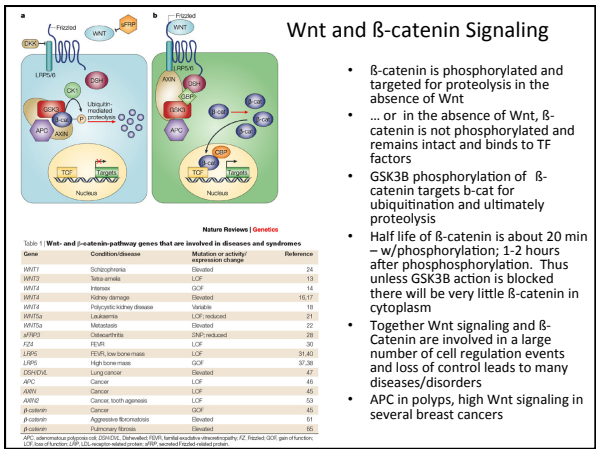
- Tumor suppressor APC mutations do not reduce ß-catenin slowing migration and causing greater proliferation and less differentiation. Allow build up of additional mutations for full development of tumor mass – multiple polyps



Wnt and ß-catenin Signaling



- ß-catenin has several signaling roles – one is via Wnt
- Wnt – extracellular GPCR 350-400 aa agonist. Family of conserved glycoproteins that are also palmitoylated for secretion and membrane association
- Wnt binds to GPCR class (Fz/frizzled family receptors) AND must bind to co-receptor lipoportein receptor-related protein (LRP). TOGETHER, Wnt signals in two distinct pathways – Canonical and noncanonical



Wnt and ß-catenin Signaling

- ß-catenin is phosphorylated and targeted for proteolysis in the absence of Wnt
- … or in the absence of Wnt, ß-catenin is not phosphorylated and remains intact and binds to TF factors
- GSK3B phosphorylation of ß-catenin targets b-cat for ubiquitination and ultimately proteolysis
- Half life of ß-catenin is about 20 min – w/phosphorylation; 1-2 hours after phosphosphorylation. Thus unless GSK3B action is blocked there will be very little ß-catenin in cytoplasm
- Together Wnt signaling and ß-Catenin are involved in a large number of cell regulation events and loss of control leads to many diseases/disorders
- APC in polyps, high Wnt signaling in several breast cancers

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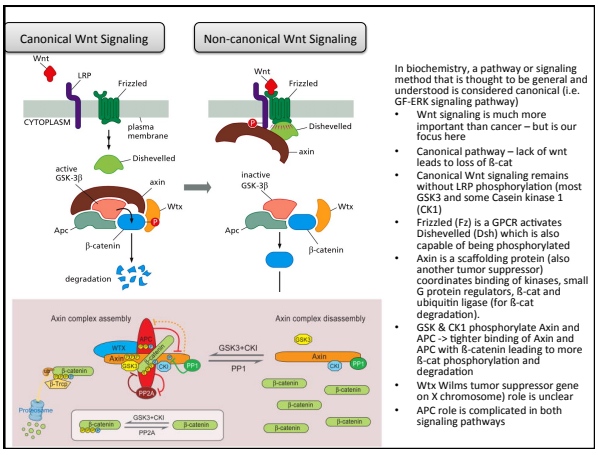
Table 1 | **Wnt- and β-catenin-pathway genes that are involved in diseases and syndromes**

Gene	Condition/disease	Mutation or activity/ expression change	Reference
WNT1	Schizophrenia	Elevated	24
WNT3	Tetra-amelia	LOF	13
WNT4	Intersex	GOF	14
WNT4	Kidney damage	Elevated	16,17
WNT4	Polycystic kidney disease	Variable	18
WNT5a	Leukaemia	LOF; reduced	21
WNT5a	Metastasis	Elevated	22
sFRP3	Osteoarthritis	SNP; reduced	28
FZD	FEVR	LOF	30
LRP5	FEVR, low bone mass	LOF	31,40
LRP5	High bone mass	GOF	37,38
DSH/DVL	Lung cancer	Elevated	47
APC	Cancer	LOF	48
AXIN	Cancer	LOF	45
AXIN2	Cancer, tooth agenesis	LOF	53
β-catenin	Cancer	GOF	45
β-catenin	Aggressive fibromatosis	Elevated	61
β-catenin	Pulmonary fibrosis	Elevated	65

APC, adenomatous polyposis coli; DSH/DVL, Dishevelled; FEVR, familial exudative vitreoretinopathy; FZ, Frizzled; GOF, gain of function; LOF, loss of function; LRP, LDL-receptor-related protein; sFRP, secreted Frizzled-related protein

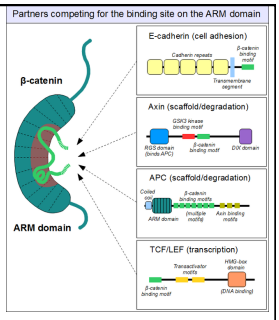


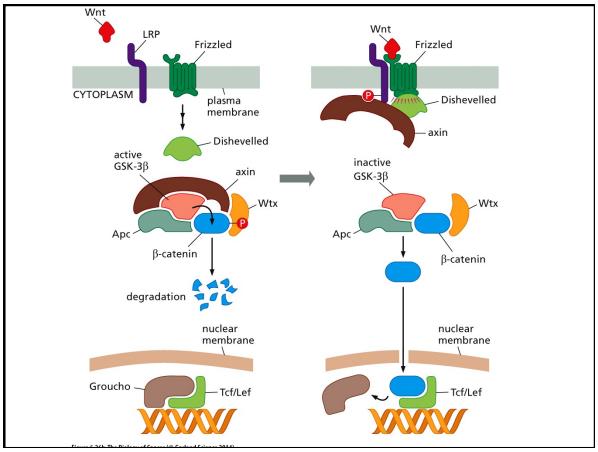
In biochemistry, a pathway or signaling method that is thought to be general and understood is considered canonical (i.e. GF-ERK signaling pathway)

- Wnt signaling is much more important than cancer – but is our focus here
- Canonical pathway – lack of wnt leads to loss of ß-cat
- Canonical Wnt signaling remains without LRP phosphorylation (most GSK3 and some Casein kinase 1 (CK1)
- Frizzled (Fz) is a GPCR activates Dishevelled (Dsh) which is also capable of being phosphorylated
- Axin is a scaffolding protein (also another tumor suppressor) coordinates binding of kinases, small G protein regulators, ß-cat and ubiquitin ligase (for ß-cat degradation).
- GSK & CK1 phosphorylate Axin and APC -> tighter binding of Axin and APC with ß-catenin leading to more ß-cat phosphorylation and degradation
- Wtx Wilms tumor suppressor gene on X chromosome) role is unclear
- APC role is complicated in both signaling pathways

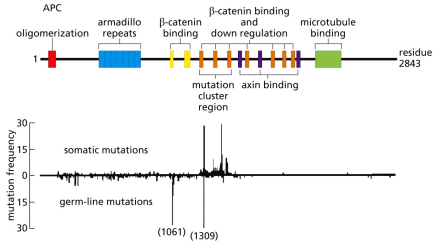
APC

- Adenomatous polyposis coli gene – tumor suppressor protein encoded by APC gene.
- Large protein that complexes with axin and conductin to bring ß-catinin to GSK3B and CK1.
- APC forms oligomers, an armadillo region (ARM - small repeats forming hairpin turns and helixes) and binding sites for other proteins.
- Dimers of APC are critical for APC function. APC mutants can bind and block wild-type APC (dominant tumor suppressor function)
- APC forms multiprotein complex to phosphorylate ß-catenin.
- In Wnt activated crypt cells – APC is not expressed leaving ß-cantenin un-degraded for TF binding/activation





Tumor Suppressor APC Gene Inactivation



- Most common mutations are mapped to regulatory sites of APC protein -> truncated APC or non-protein binding APC
- Some colon polyps show hypermethylation in APC gene decreasing APC expression
- Results in accumulation of ß-catenin in 90% of sporadic colon carcinomas
- Exact role is not as clear as this but mutations help our understanding of role as a suppressor