Dose Response 2013
UMass Amherst, April 24, 2013

Application of the Dysregulated Adaptive Hyperplasia (DAH) Tumorigenesis Model to Estimate Low-Dose Dibenzo[*a*,*l*]pyrene Tumor Risk

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Contending theories of tumorigenesis each posit specific types of events as pivotal

- Multistage somatic-oncogene mutation, with clonal expansion of premalignant cells
- Chronic oxidative stress
- Chronic inflammation/infection and its microenvironment
- Defective wound healing
- Aberrant DNA methylation
- Autocatalytic aneuploidy and consequent genomic instability
- MicroRNA (miRNA) dysregulation

Since 1976, USEPA has cited only the somatic-mutation theory as its key basis for linear-no-threshold (LNT) risk extrapolation for chemical carcinogens

- Multistage somatic-oncogene mutation, with clonal expansion of premalignant cells
- Chronic oxidative stress
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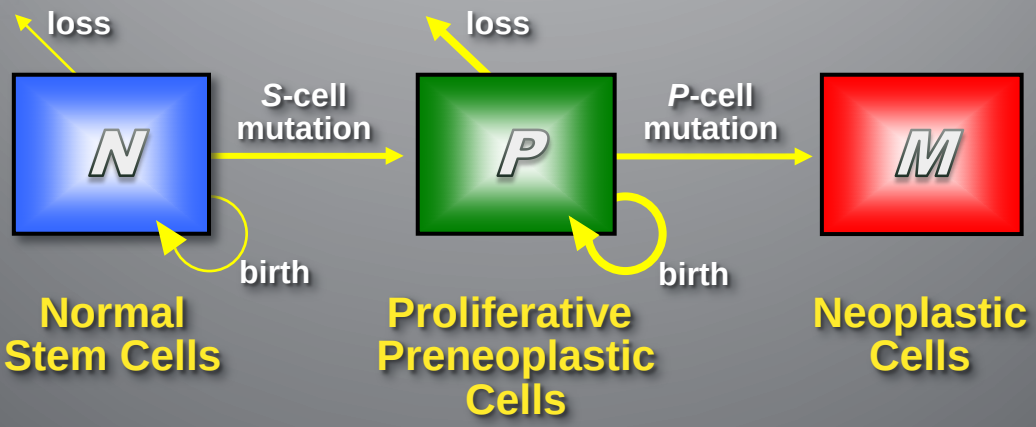
- MicroRNA (miRNA) dysregulation

Multistage somatic-mutation/proliferation: 2-stage stochastic clonal-expansion model



Source: Armitage & Doll 1957; MVK ~1979+

- Insertion of a few genes is sufficient to transform cultured normal human epithelial cells or fibroblasts into malignant cancer cells
- Proliferative premalignant foci identified immunohisto-chemically appear to evolve into adjacent, further-transformed neoplasia

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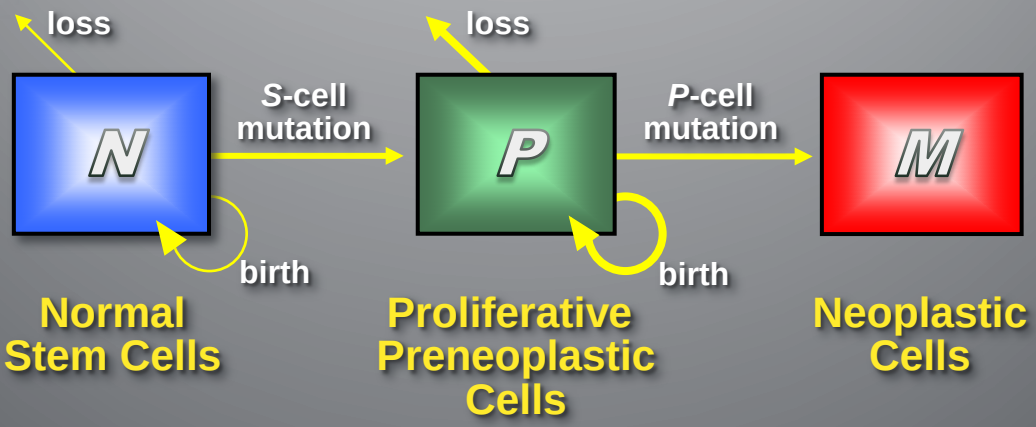
Multistage somatic-mutation/proliferation: 2-stage stochastic clonal-expansion model



Source: Armitage & Doll 1957; MVK ~1979+

- Many suspected oncogenes are recessive, not dominant
- Activated dominant *ras* is found in normal tissues
- Stably benign vs. malignant tumor phenotypes
- Typically, cancer-cell genomes sporadically (not gradually) accumulate 10^3–10^4 somatic mutations
- Cancer cells tend to be aneuploid with translocations

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The most recent cancer theory, concerning microRNA (miRNA), now generates intense research activity with demonstrated clinical relevance

- Multistage somatic-oncogene mutation, with clonal expansion of premalignant cells
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Introduction to miRNA

- miRNAs are ~22-nucleotide-size interference-RNAs that powerfully augment the Watson-Crick "central dogma" of genome expression:

$$\text{Gene} \rightarrow \text{mRNA}^* \rightarrow \text{protein}$$

- Each miRNA efficiently suppresses translation of all mRNAs that include a subsequence that complements (and so is "targeted" by) that miRNA
- There are 100s of miRNAs, each of which may target up to 100s of mRNAs

*mRNA = messenger RNA

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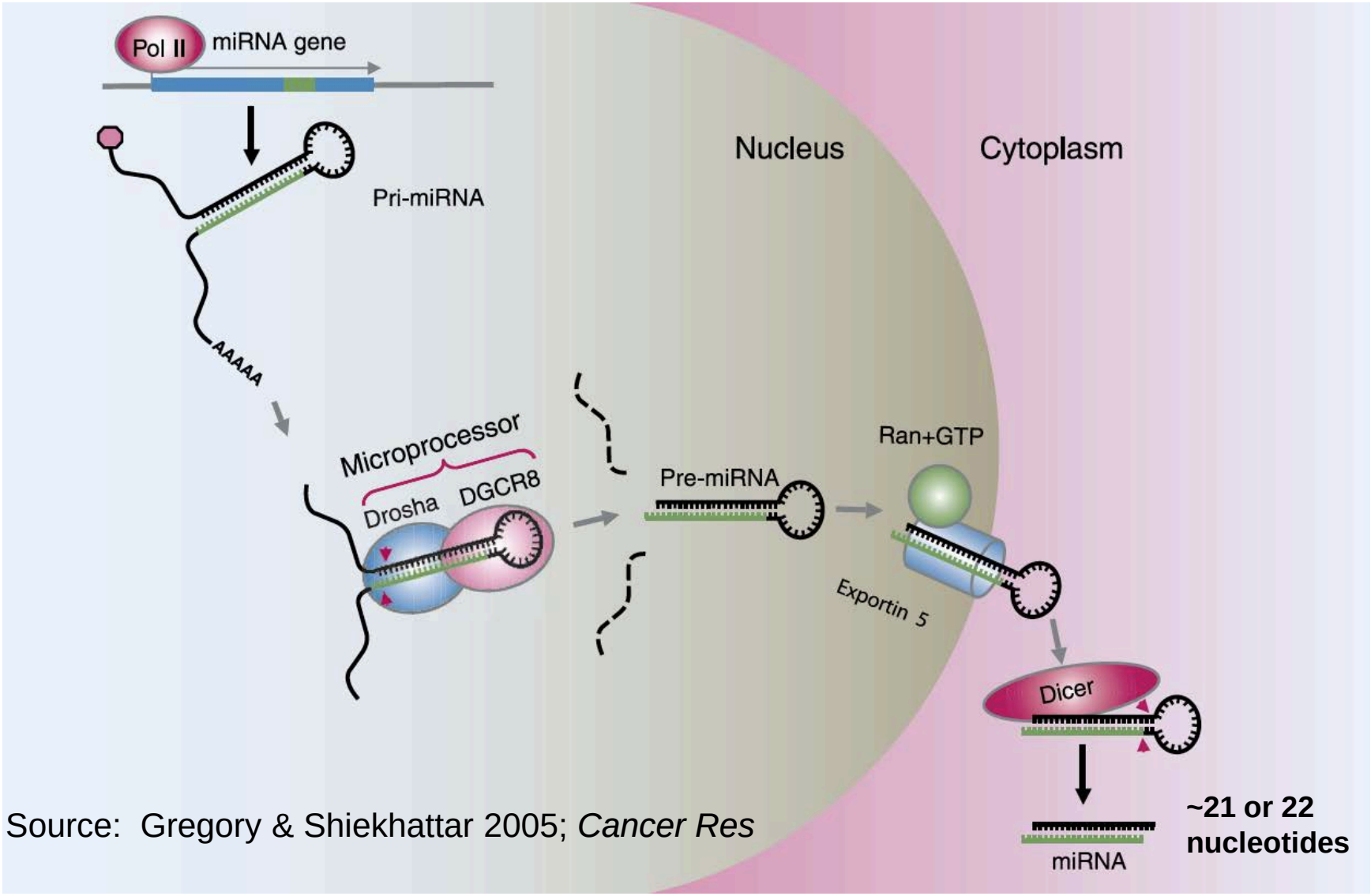
Introduction to miRNA *(continued)*

- Nature thus implements a "bar-coding" system by which genes and their mRNAs are targeted for efficient suppression by small miRNAs
- Suppressed promoter genes can induce the expression of other genes
- miRNA expression profiles may affect and be influenced by patterns of DNA methylation, and so may be maintained epigenetically
- miRNA-expression profiles thus efficiently orchestrate complex patterns of genome expression

Introduction to miRNA *(continued)*

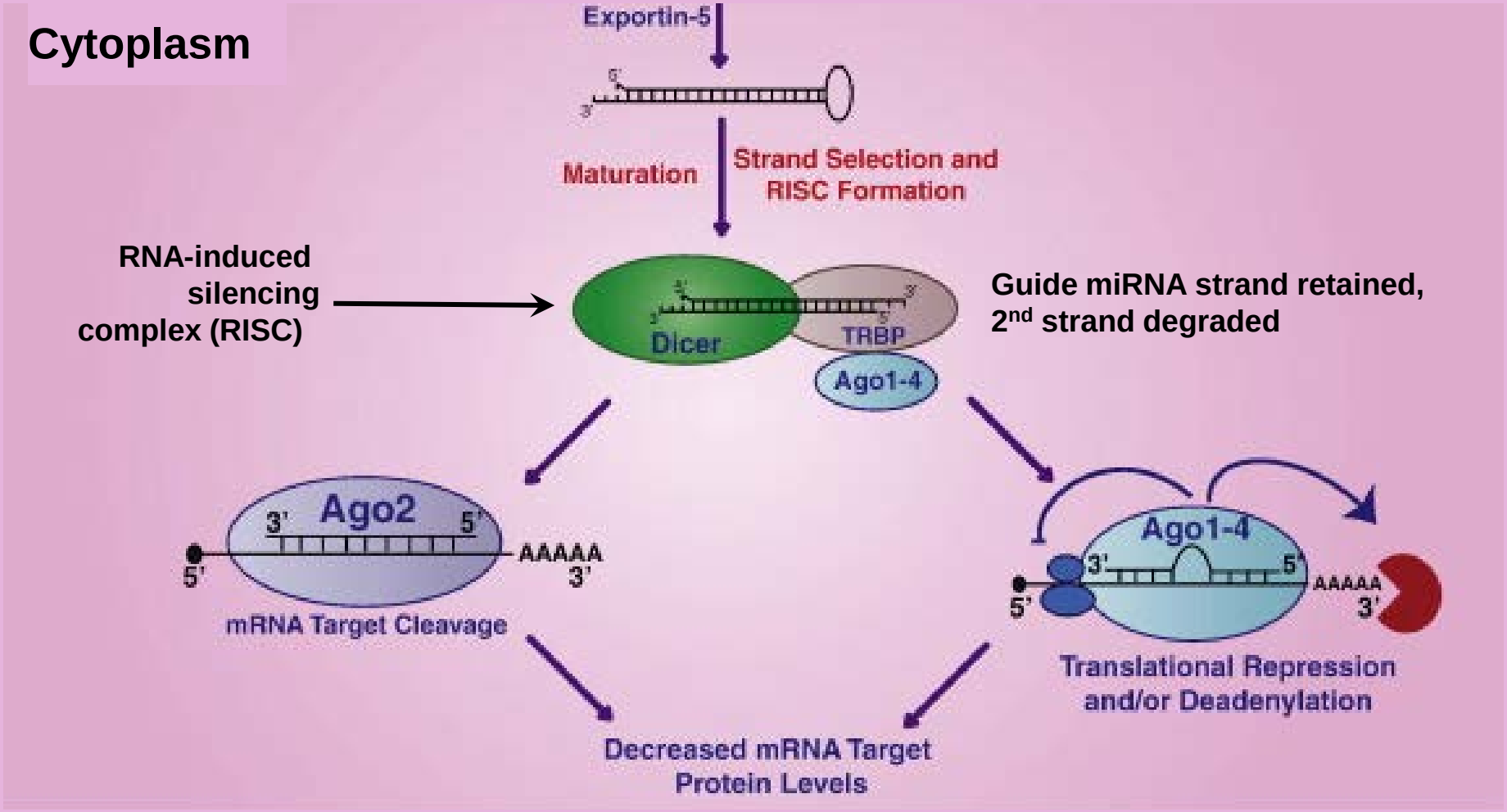
- The first miRNA was discovered by Victor Ambros and his lab in 1993, in a pathway controlling development in the nematode *C. elegans*
- miRNAs were later found to be highly conserved evolutionarily in all plant and animal cells
- Ambros got the Lasker Prize in 2008 for discovering and exploring miRNA functions
- Craig Mello (from Ambros' Lab) and Andrew Fire got a Nobel Prize in 2006 for their related discovery of RNA interference

miRNA precursors made in the nucleus are processed there, actively exported to the cytoplasm, then further processed…



Source: Gregory & Shiekhattar 2005; *Cancer Res*

RISC-bound miRNAs in cytoplasm actively suppress or prevent targeted mRNAs from being translated into protein



Source: Elton et al. 2011; *Life Sci*

miRNAs have critical regulatory functions

- **Embryogenesis and development**
- **Adult cell and tissue responses to**
  - Stress
  - Viral, bacterial, fungal, and parasitic infections*
  - Other (cardiovascular, neoplastic) pathologies*

*See, e.g., http://www.mir2disease.org

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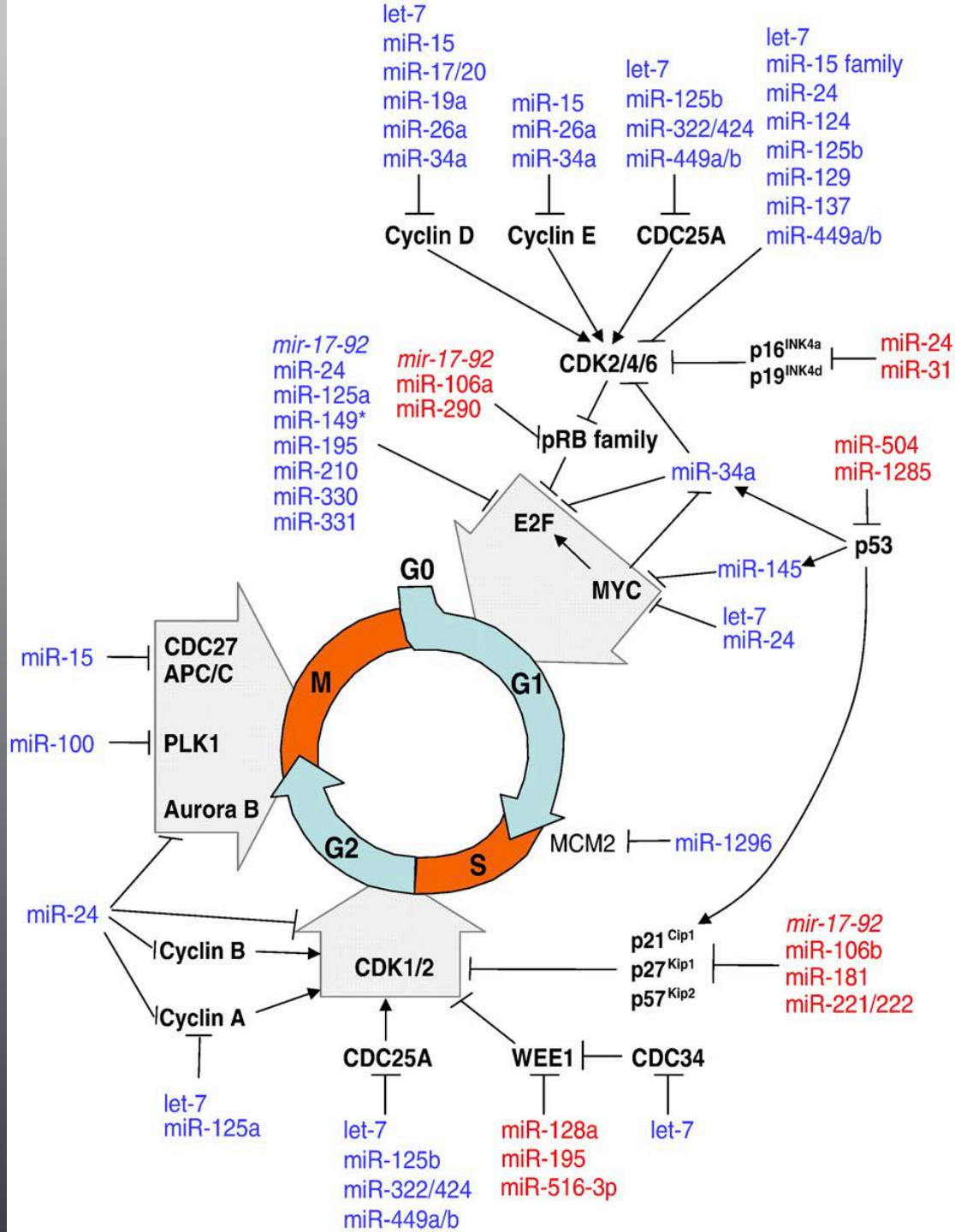
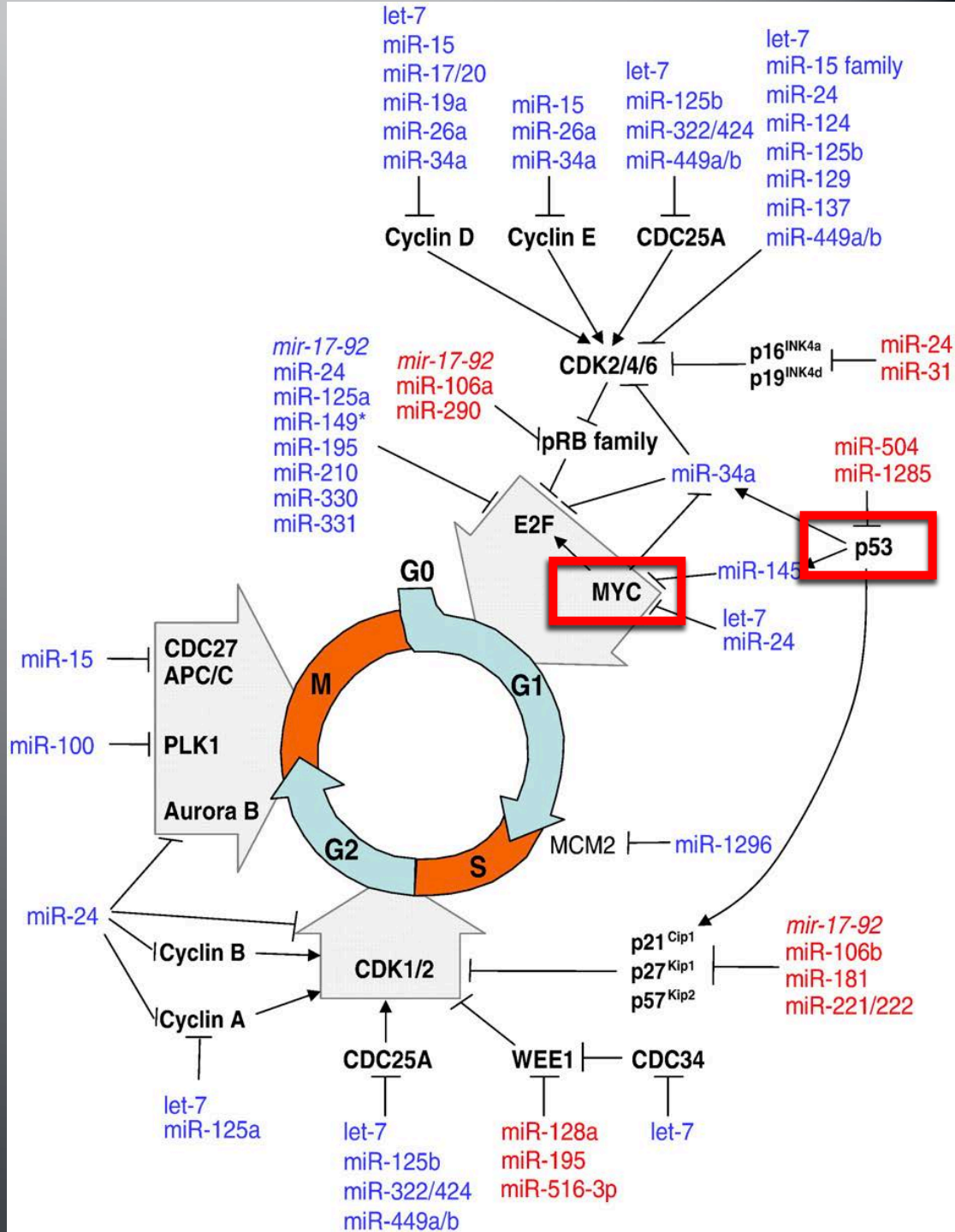
miRNAs regulate the cell cycle



Source: Bueno & Malumbres 2011; *Biochim Biophys Acta*

miRNAs regulate the cell cycle

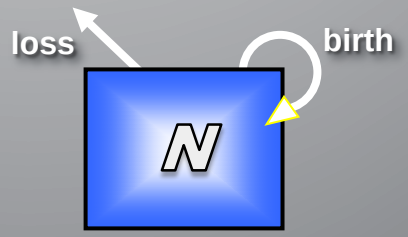


Source: Bueno & Malumbres 2011; *Biochim Biophys Acta*

R-type Adaptive Hyperplasia: Regenerative/repair response to cell killing and tissue damage caused by infection, wounding, or disease

Stem Cell Population

Normal



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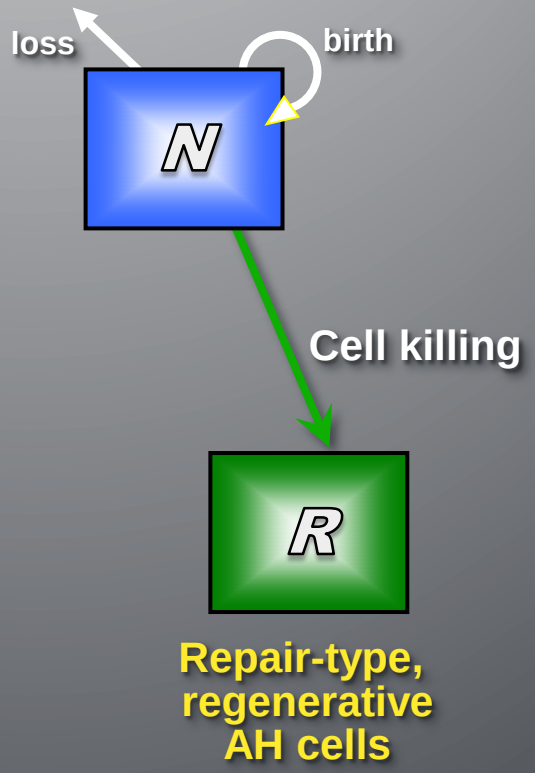
R-type Adaptive Hyperplasia: Regenerative/repair response to cell killing and tissue damage caused by infection, trauma, or disease

Stem Cell Population

Normal



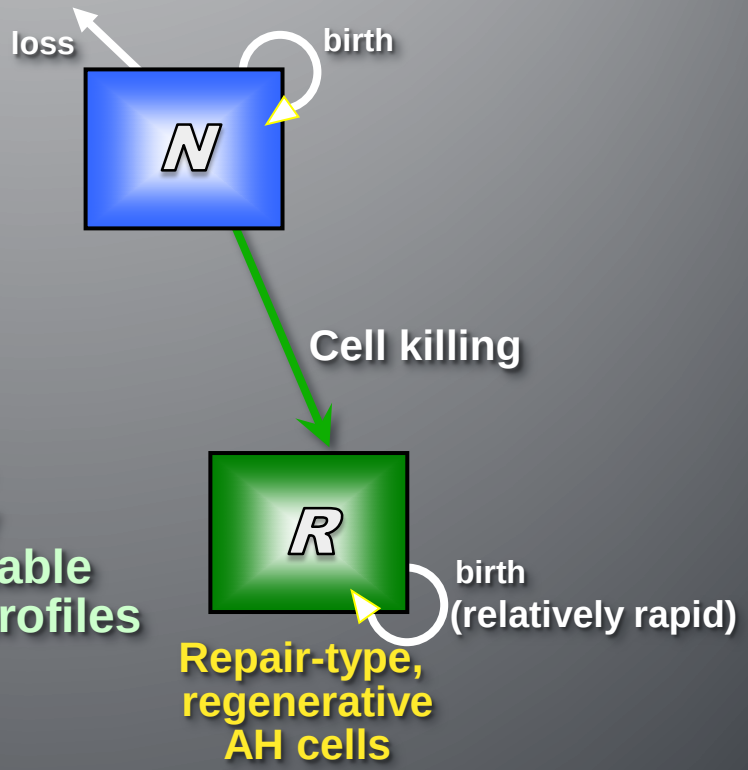
Adaptive Hyperplasia (AH)

Exponent

R-type Adaptive Hyperplasia: Regenerative/repair response to cell killing and tissue damage caused by infection, trauma, or disease

Stem Cell Population

Normal



Adaptive Hyperplasia (AH)

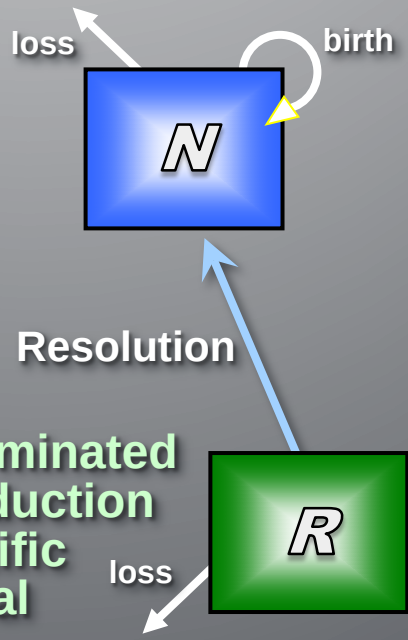
AH states are maintained by epigenetically heritable miRNA-expression profiles

Exponent

Resolution of R-type adaptive hyperplasia (AH) is mediated by a tissue-specific signal

Stem Cell Population

Normal



Adaptive Hyperplasia (AH)

AH state normally terminated by successful transduction of a tissue-specific resolution signal

Repair-type, regenerative AH cells

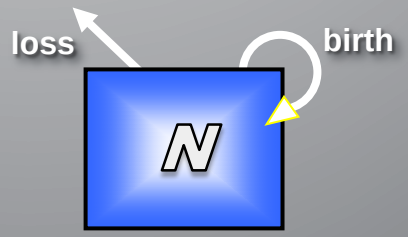
IL-6	→	B-cell, macrophage differentiation
IL-21	→	B-cell apoptosis
GDF-5	→	B-cell growth arrest
FGF	→	chondrocyte growth arrest
TGF-β	→	epithelial cell growth arrest
Ceramide	→	apoptosis (many cell types)
IGFBP-3	→	growth inhibition or apoptosis
OSM, EGF block	→	mammary epithelial growth arrest

Exponent

Successful resolution of R-type AH returns damaged tissue to its initial state

Stem Cell Population

Normal

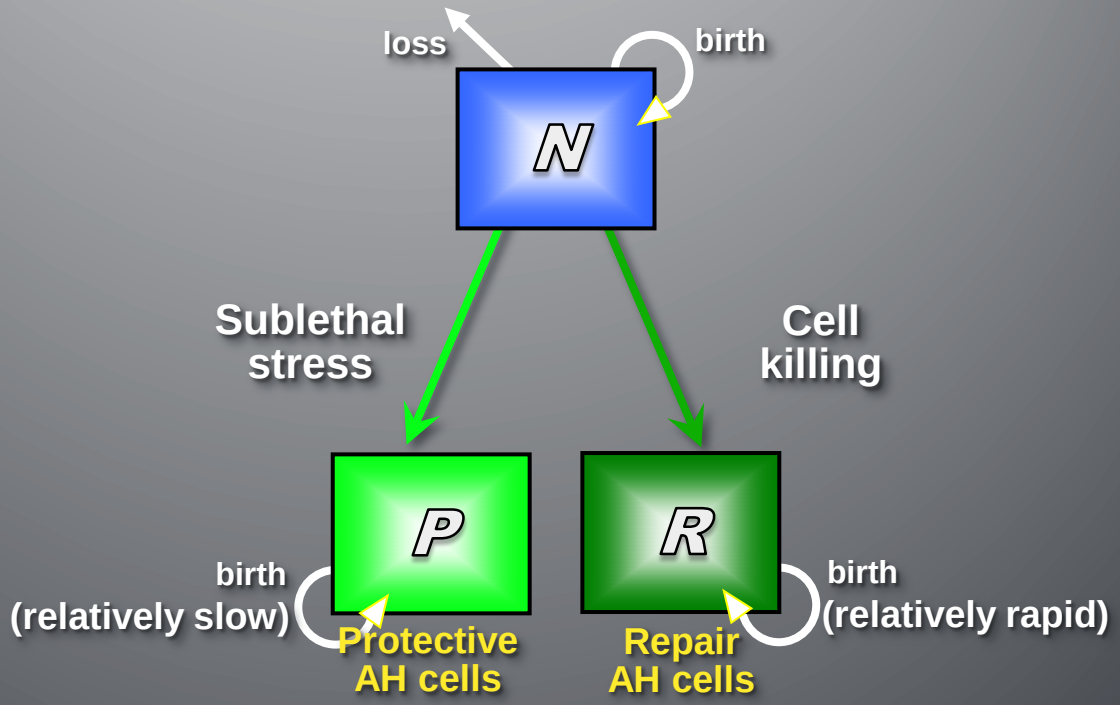


P-type Adaptive Hyperplasia: Protective proliferative response to mechanical, metabolic, or toxic stress

Stem Cell Population

Normal

Adaptive Hyperplasia (AH)



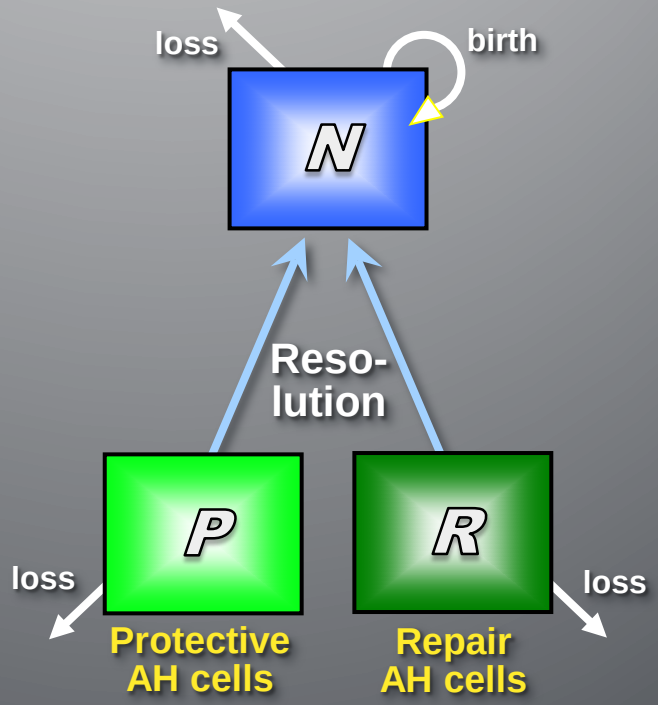
Exponent

P-type Adaptive Hyperplasia: Protective proliferative response to mechanical, metabolic, or toxic stress

Stem Cell Population

Normal

Adaptive Hyperplasia (AH)



Exponent

Dysregulated miRNA profiles are specific to tumor types and tumor prognosis

miRNA	Cell cycle regulator	Down (↓), Up (↑), or De (~) -regulation in cancers
let-7 family	CDC25A, CDC34, CDK4, CDK6, Cyclin A, D1, D2, D3, c-MYC	↓ in leukemias, lymphomas, melanoma, lung, breast, gastric, gastric, pancreatic, pituitary, ovarian, kidney, prostate & colon cancer, hepatocellular carcinoma, multiple myeloma
miR-15 family (miR-15, 16, 195)	CDC27, CDK6, Cyclin D1, D3, E1, E2F3, WEE1	↓ in CLL, DLBCL, multiple myeloma, pituitary adenoma, prostate, & pancreatic cancer
miR-17 family (miR17, 20, 106, 93)	Cyclin D1, E2F1, MYCN, p21Cip1, pRb family	↑ in lung and colon cancer, lymphoma, multiple myeloma, medulloblastoma; ↓ in melanoma, ovarian & breast cancer
miR-19a	CyclinD1	~ in leukemias, hepatocellular carcinoma, colorectal & lung cancer
miR-24	AURKB, CDK1, CDK4, Cyclin A2, Cyclin B, E2F2, MYC, p16INK4a	~ in in some leukemias, hepatocellular carcinoma & prostate cancer
miR-25	p57Kip2	~ in glioblastoma, hepatocellular carcinoma, colorectar, gastric, pancreatic & prostate cancer
miR-26a	Cyclins D2 & E2	↓ in leukemia, Burkitt lymphomas, glioma, pituitary, thyroid, liver, kidney, ovarian, bladder & breast cancer
miR-31	p16INK4a, p19INK4d	~ in bladder, breast, colorectal, liver, lung, pancreatic & prostate cancer
miR-34a	CDK4, CDK6, Cyclins D1 & E2, E2F1, E2F3, c-MYC	
miR-100	PLK1	~ in bladder, ovarian, pancreatic, prostate & nasopharyngeal cancer
miR-124a	CDK6	~ in ALL, CLL, medulloblastoma, hepatocellular carcinoma, & breast, colorectal & lung cancer
miR-125b	CDC25A, CDK6, Cyclin A, E2F3	~ in neuroblastoma, medulloblastoma, liver, bladder, breast & prostate cancer
miR-128a	WEE1	~ in ALL, AML, glioblastoma, pituitary adenomas & breast cancer
miR-129	CDK6	↓ in multiple tumor cell lines & primary tumors (medulloblastoma, undifferentiated gastric cancers, lung adenocarcinoma, endometrial, ovarian and bladder cancer, & colorectal & hepatocellular carcinoma)

Dysregulated miRNA profiles are specific to tumor types and tumor prognosis *(continued)*

miRNA	Cell cycle regulator	Tumor-specific down (↓), up (↑), or de (~) -regulation
miR-145	c-MYC	~ in leukemias, Burkitt lymphomas, bladder, breast, colorectal, ovarian, gastric, lung, pancreatic, prostate cancer & hepatocellular carcinoma
miR-149*	E2F1	~ in neuroblastoma
miR-155	WEE1	~ in leukemias & lymphomas, pituitary adenomas, hepatocellular carcinoma, breast, colorectal, ovarian, lung and pancreatic cancer
miR-181 family (miR-181a, b, c)	p27Kip1	~ in leukemias, glioblastoma, hepatocellular carcinoma, breast, colorectal, lung, pancreatic & prostate cancer
miR-210	E2F3	~ in leukemias, lymphomas, glioblastoma, breast, kidney, lung, pancreatic, prostate & ovarian cancer
miR-221 family (miR-221, 222)	27Kip1, p57Kip2	~ in leukemias, glioblastoma, breast, pancreatic, prostate, ovarian, bladder, and gastric cancer, melanoma and hepatocellular carcinoma
miR-330	E2F1	↓ in follicular lymphoma, oral squamous cell carcinoma & prostate cancer
miR-331-3p	E2F1	~ in human gastric cancer
miR-322/424, miR-503	CDC25A	↓ or ~ in in some leukemias, kidney, ovarian & pancreatic cancer, and in retinoblastoma & prostate cancer
miR-449a/449b	CDC25A, CDK6	↓ in prostate cancer
miR-516a-3p	WEE1	↑ in breast cancer & in pituitary adenomas
miR-1296	MCM2	~ in prostate cancer

Source: Bueno & Malumbres 2011; *Biochim Biophys Acta*

A new theory of tumorigenesis: Dysregulated Adaptive Hyperplasia (DAH)

- P- and R-type AH cells share key features of benign and malignant tumor cells, respectively
- Neoplastic transformation of AH cells should be relatively efficient, compared to other pathways
- The DAH theory of tumorigenesis posits that separate, efficient pathways to benign or malignant tumors arise by mutation-induced failure to resolve P- or R-type AH, respectively

Bogen 2012; *Med Hypoth* (in press)

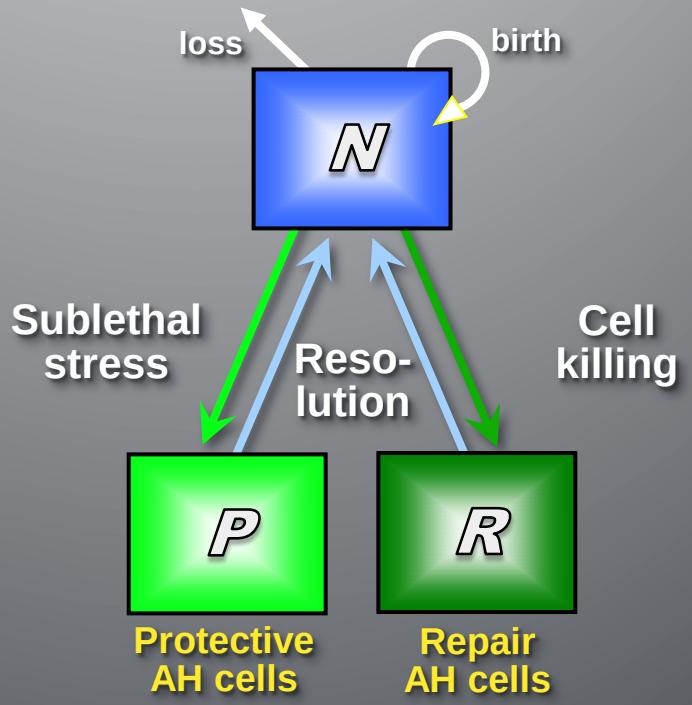
Exponent

What happens if a dominant somatic mutation blocks the transduction of a signal to resolve P- or R-type AH?

Stem Cell Population

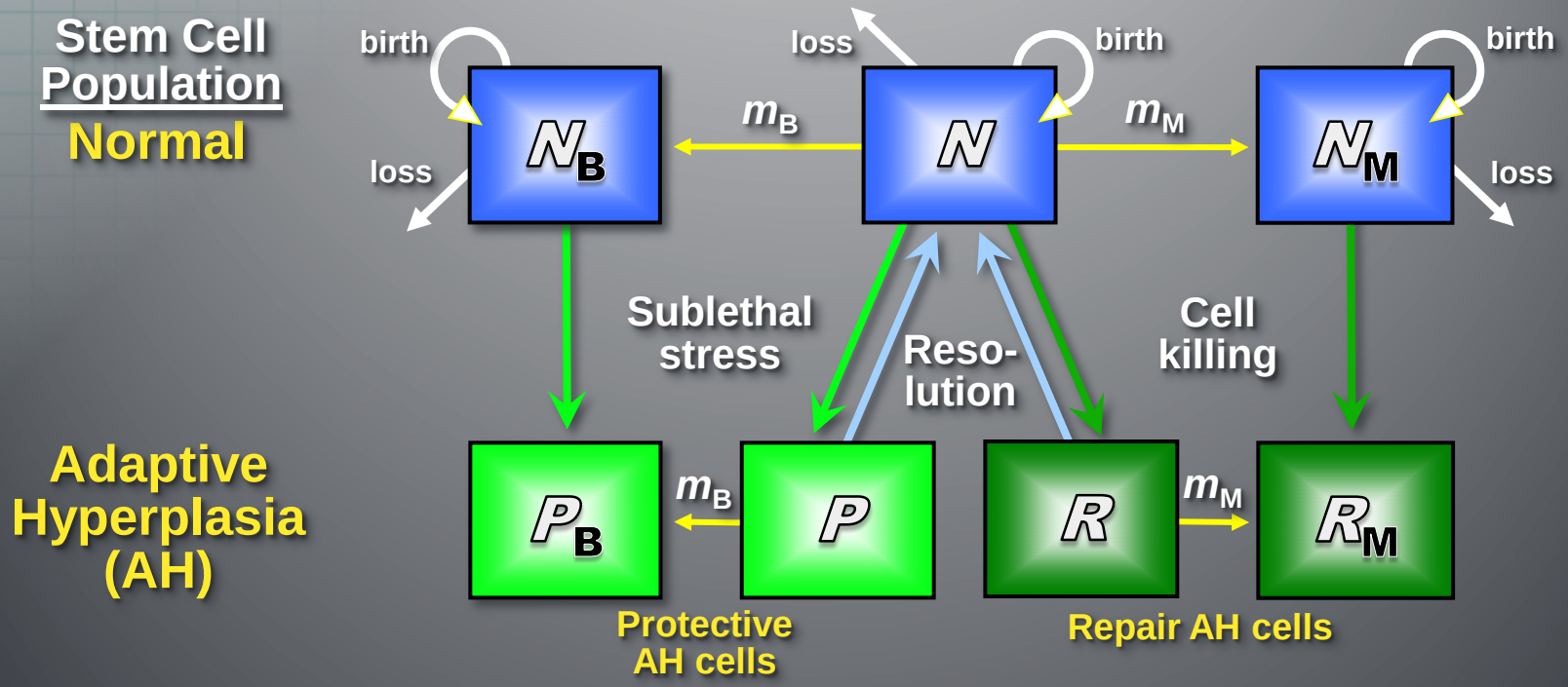
Normal

Adaptive Hyperplasia (AH)



Exponent

Inability to resolve P- or R-type AH is posited to arise by a P- or R-specific dominant mutation of normal or AH stem cells



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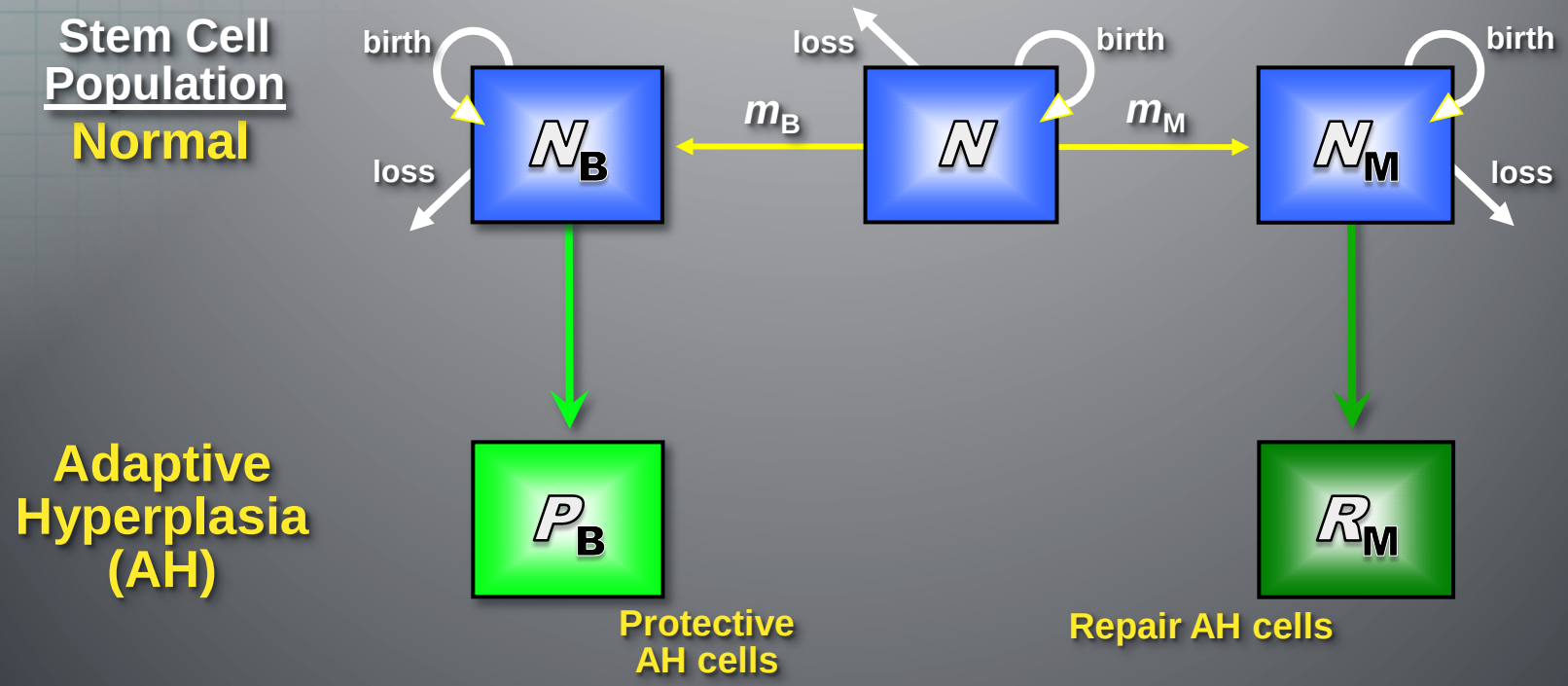
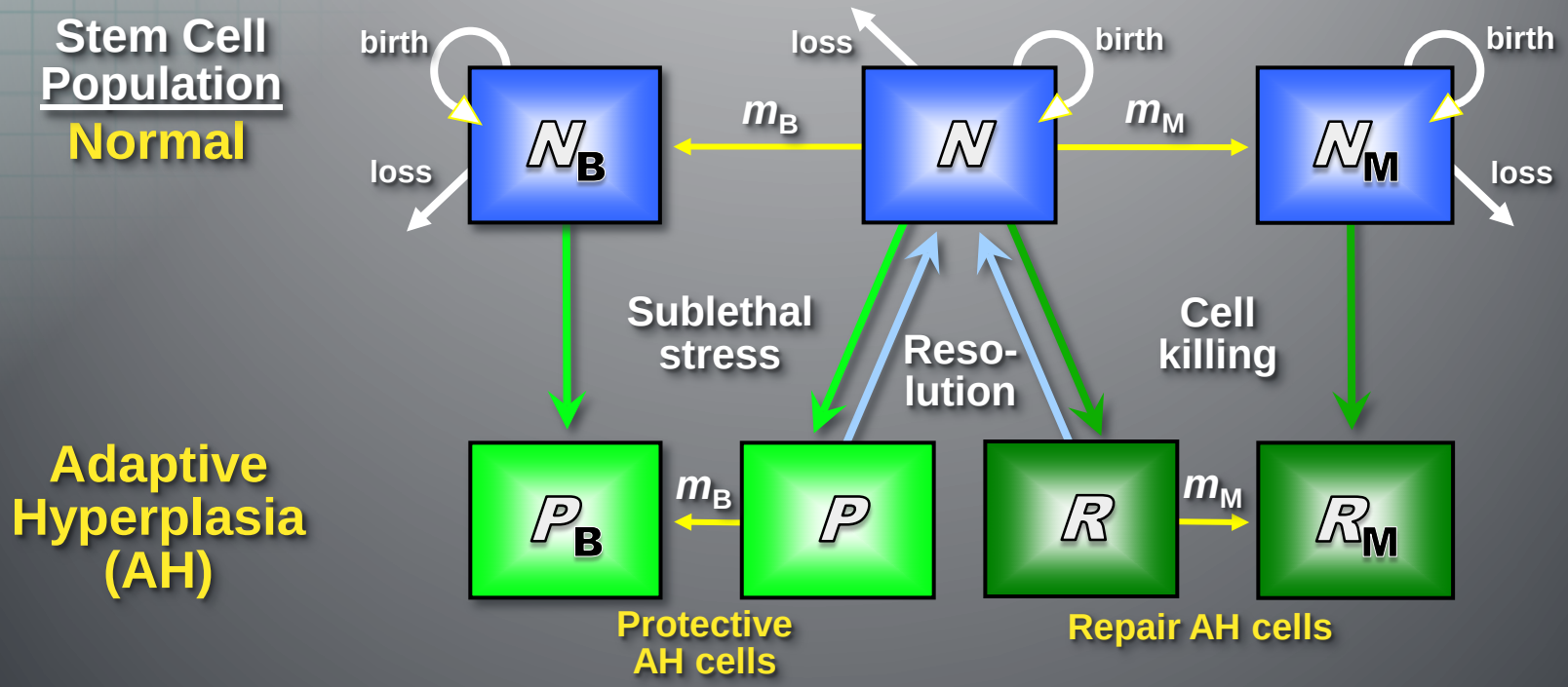
In the absence of stress-induced AH, mutated AH cells persist, and so accumulate over time



Exponent

Additional stress-induced AH adds most efficiently to the population of mutated AH cells



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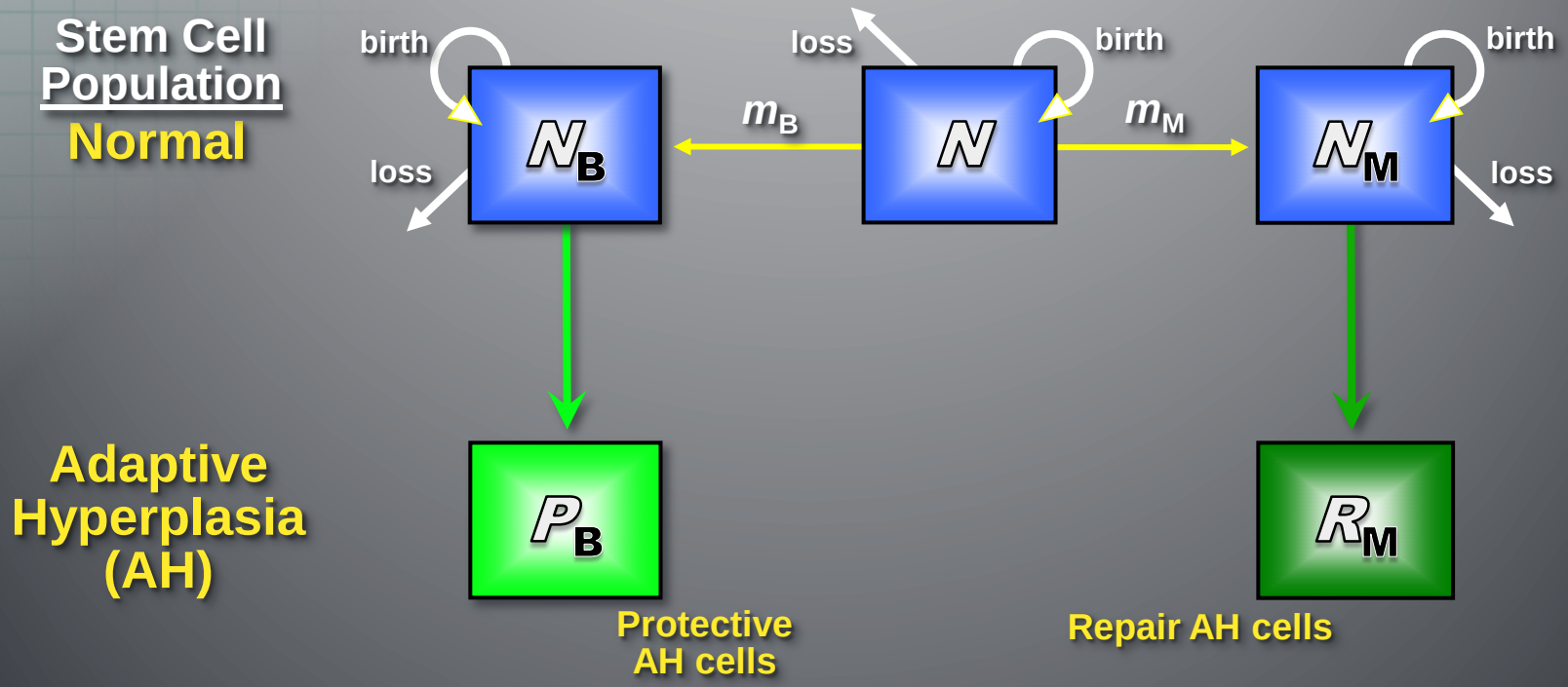

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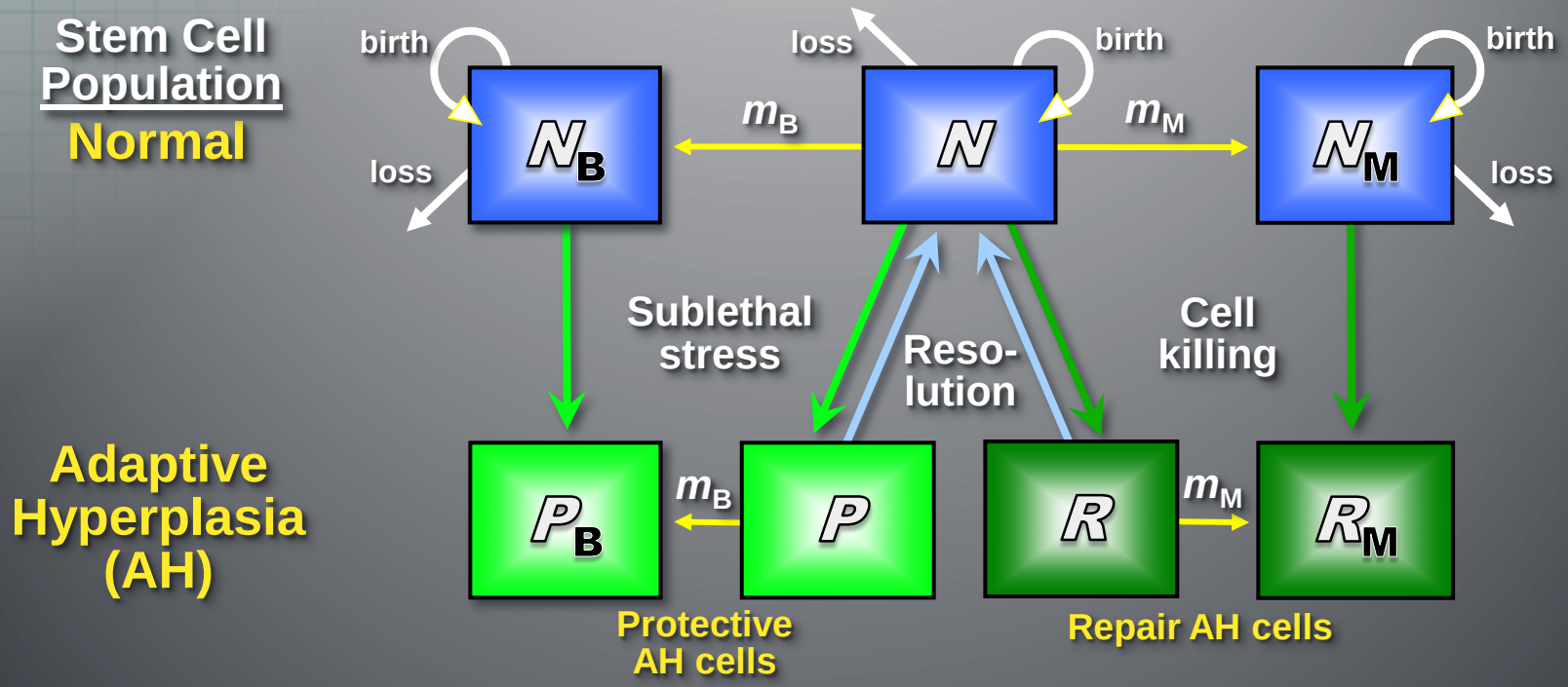

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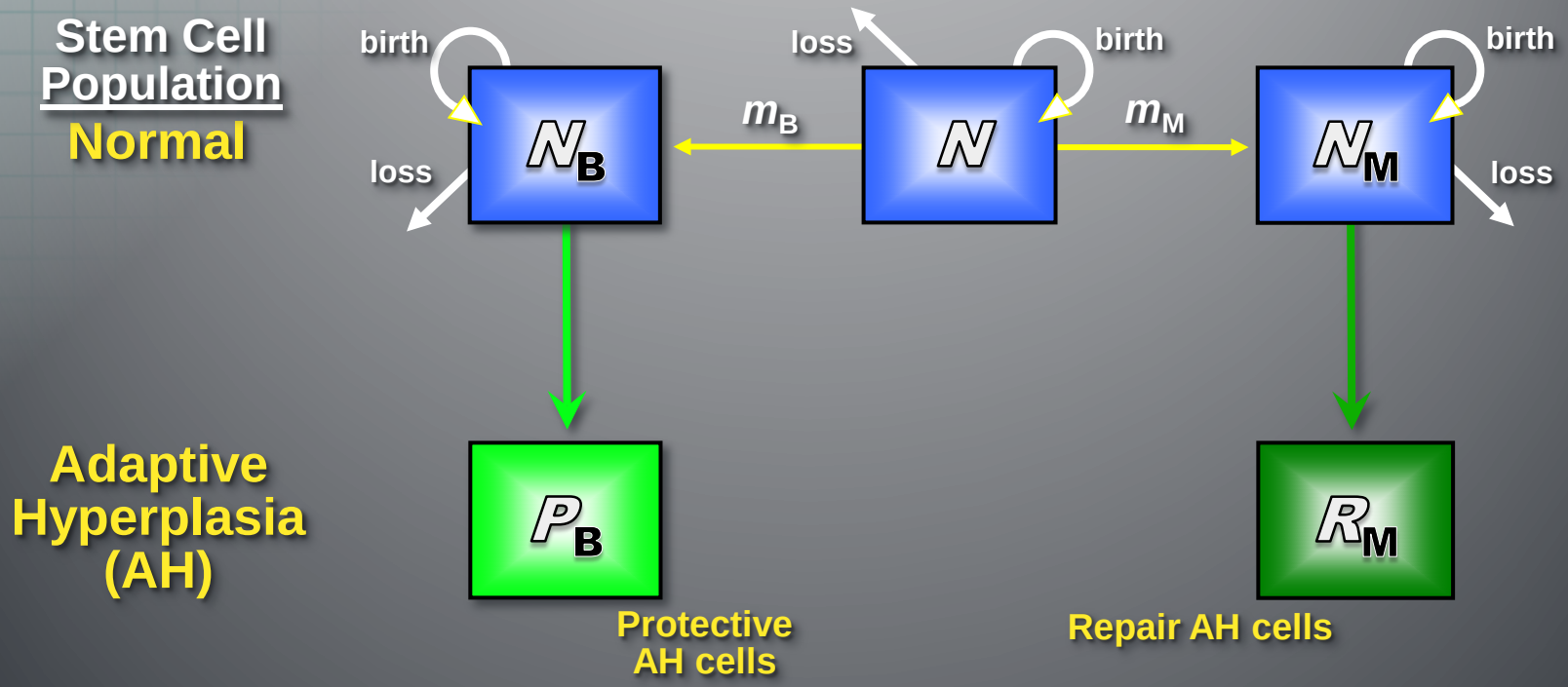
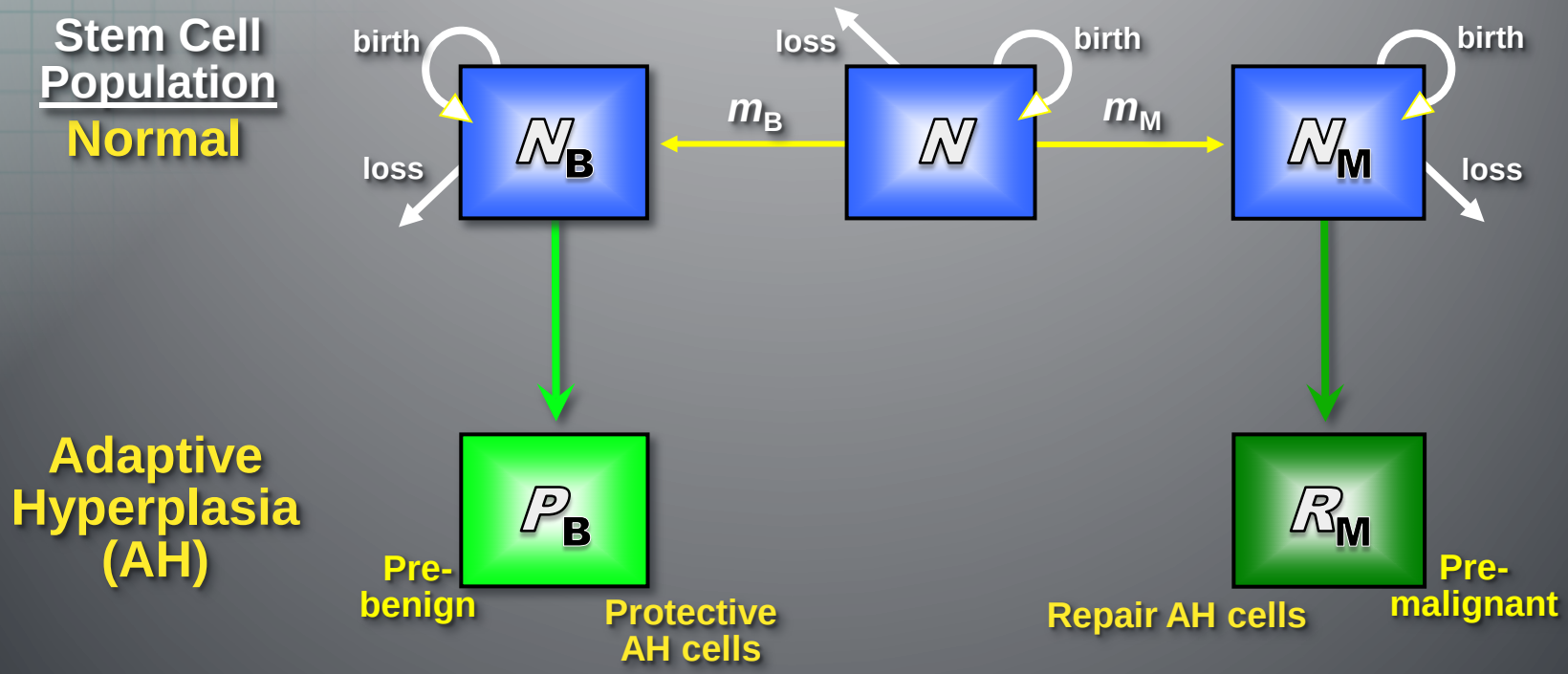
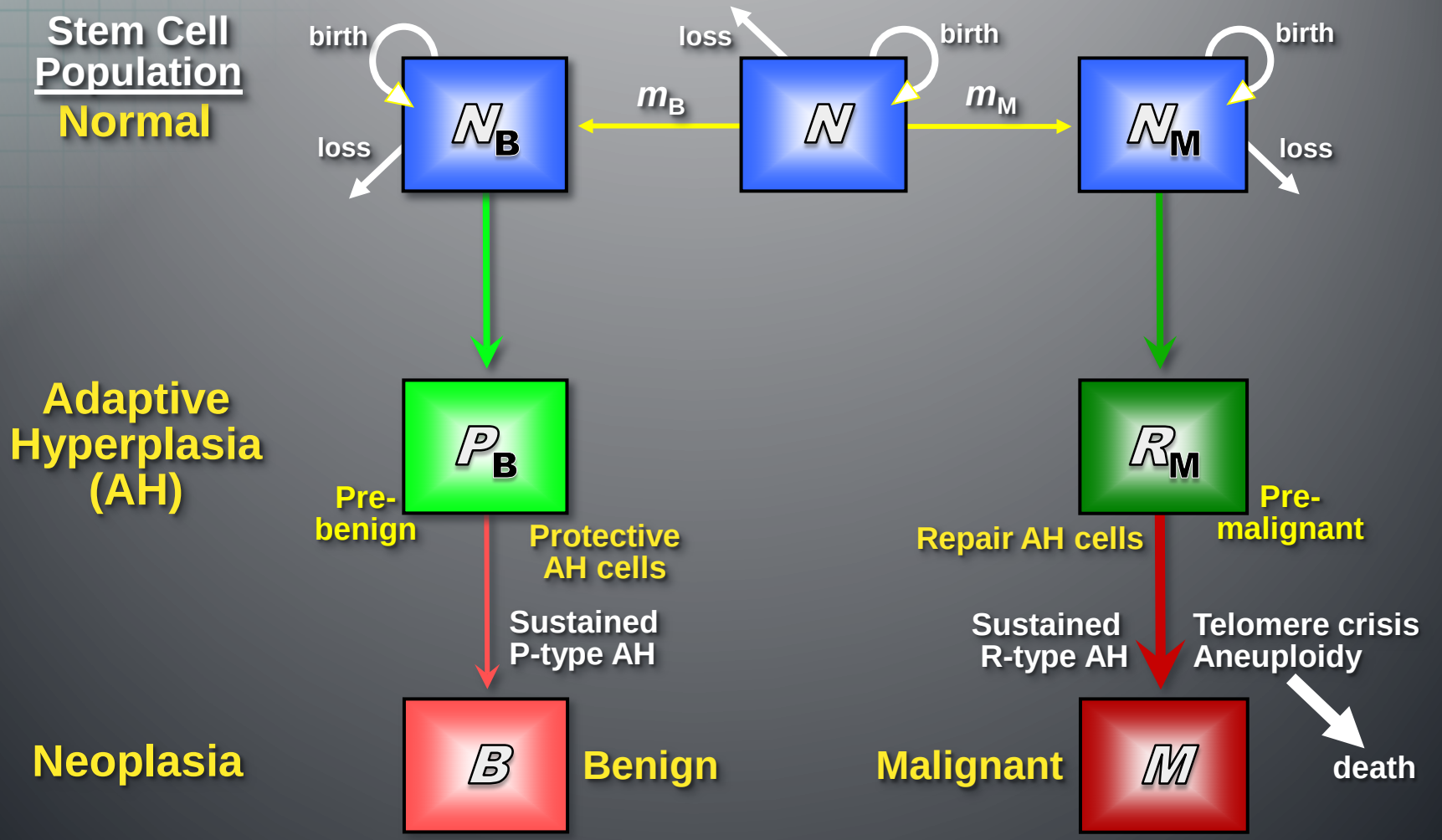


As mutated P- and R-type AH cells accumulate and proliferate clonally, they form “pre-benign” and premalignant cell foci, respectively …



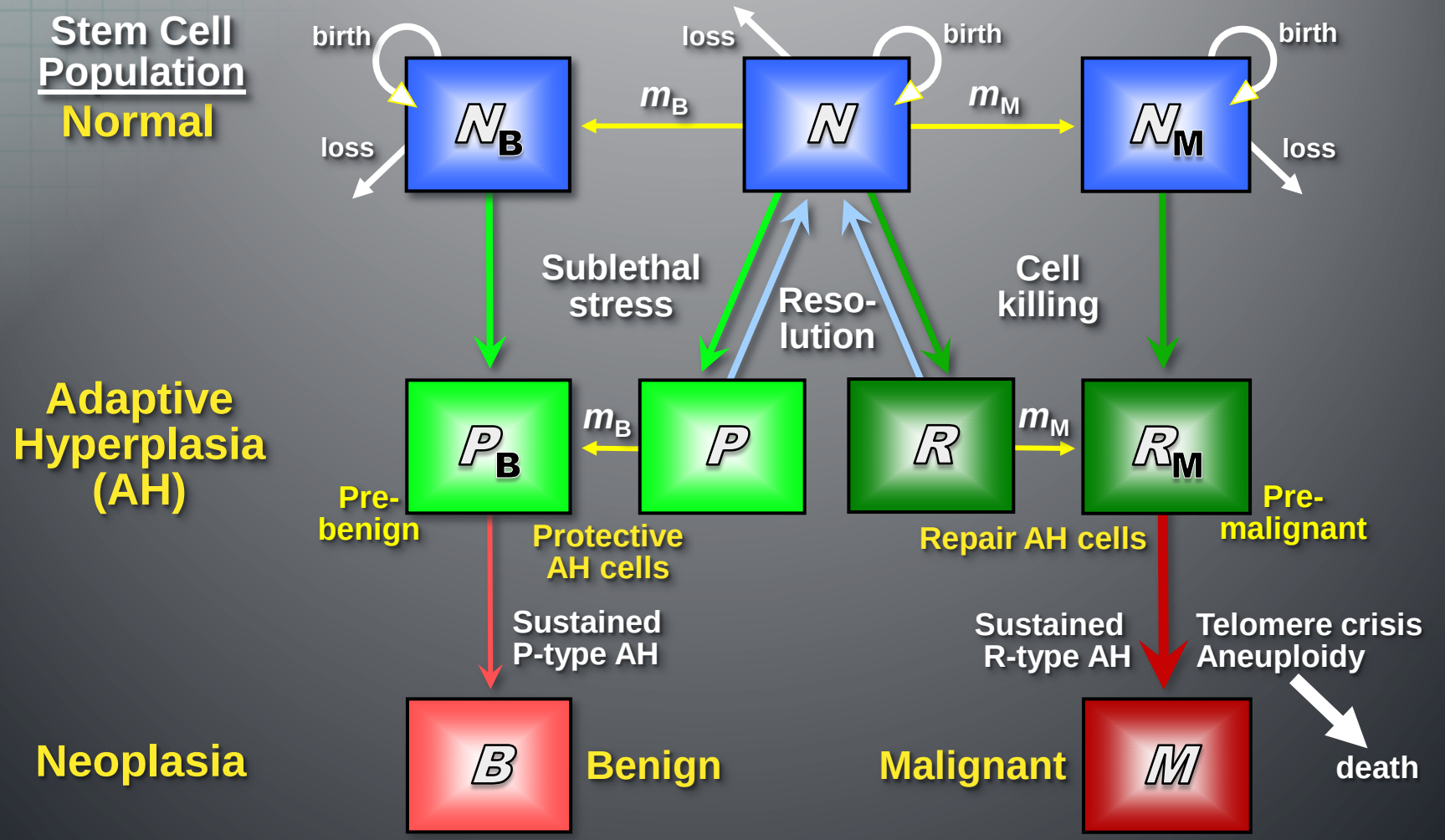
Exponent

… which form efficient dysregulated adaptive hyperplasia (DAH) pathways of tumorigenesis



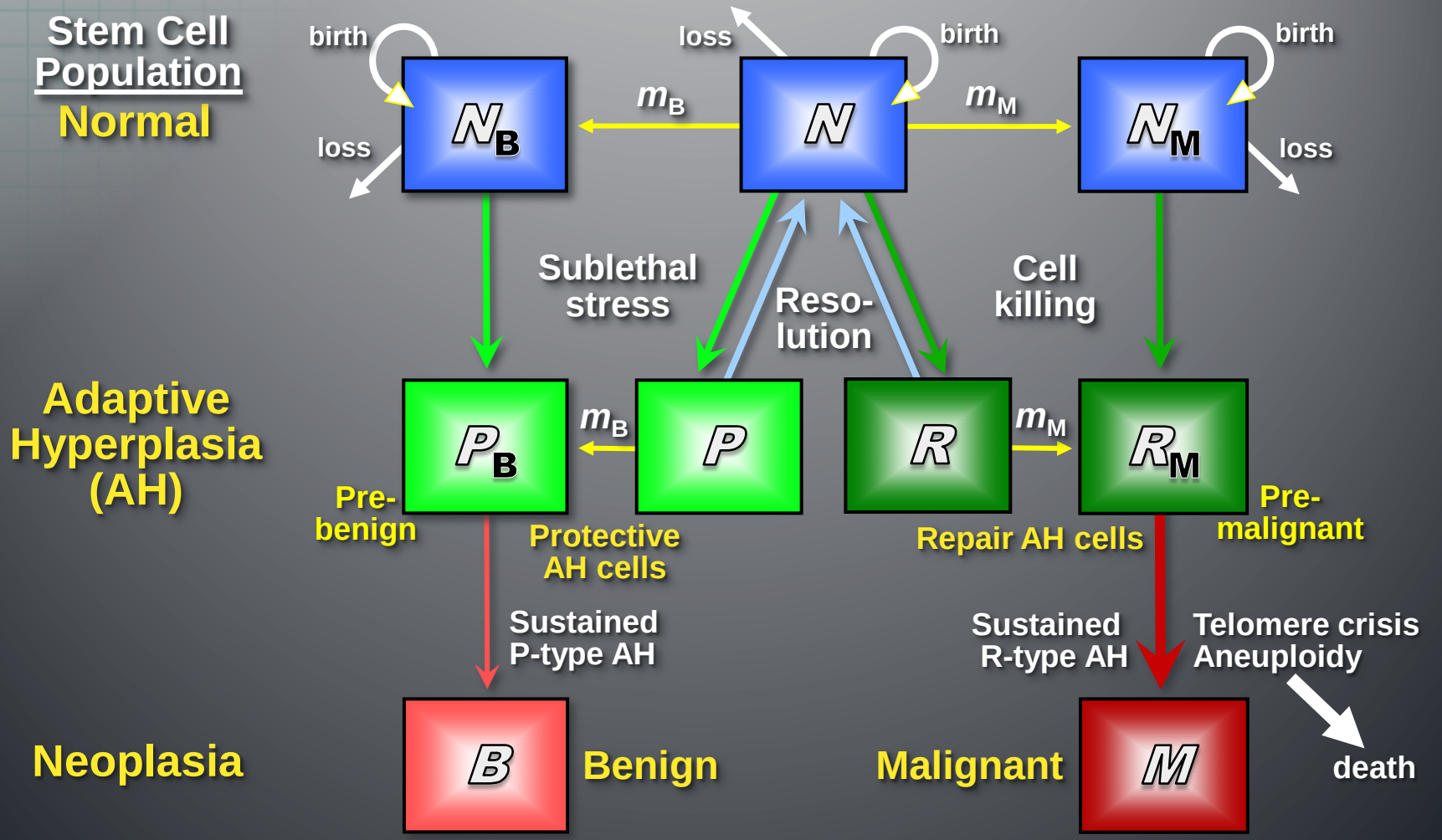
Exponent

DAH tumorigenesis is relatively efficient, because it requires only one critical mutation



Exponent

AH cell populations that drive DAH tumorigenesis typically remain small, but occasionally expand then shrink over a lifetime



Bogen 2013; *Med Hypotheses*

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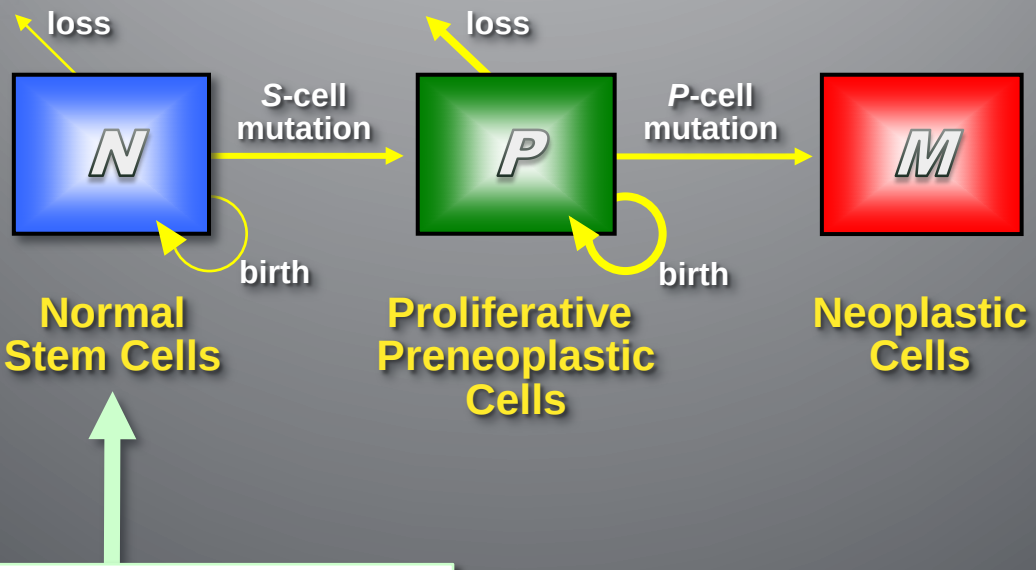
In contrast, cell populations that feed tumorigenesis under the somatic-mutation/proliferation model are typically assumed to be large and stable in adulthood

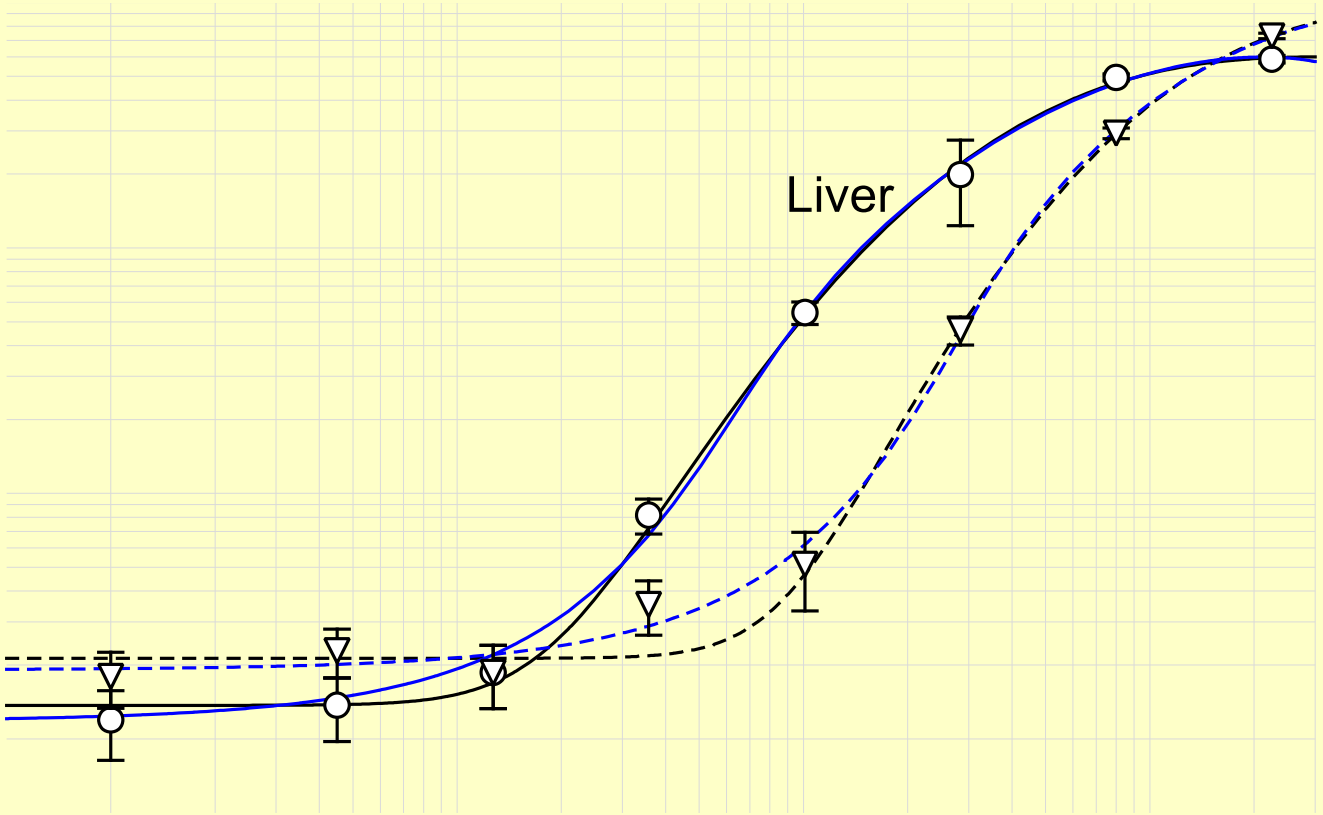


Typically approximately constant in adults (except, e.g., in female breast epithelium, as of menopause)

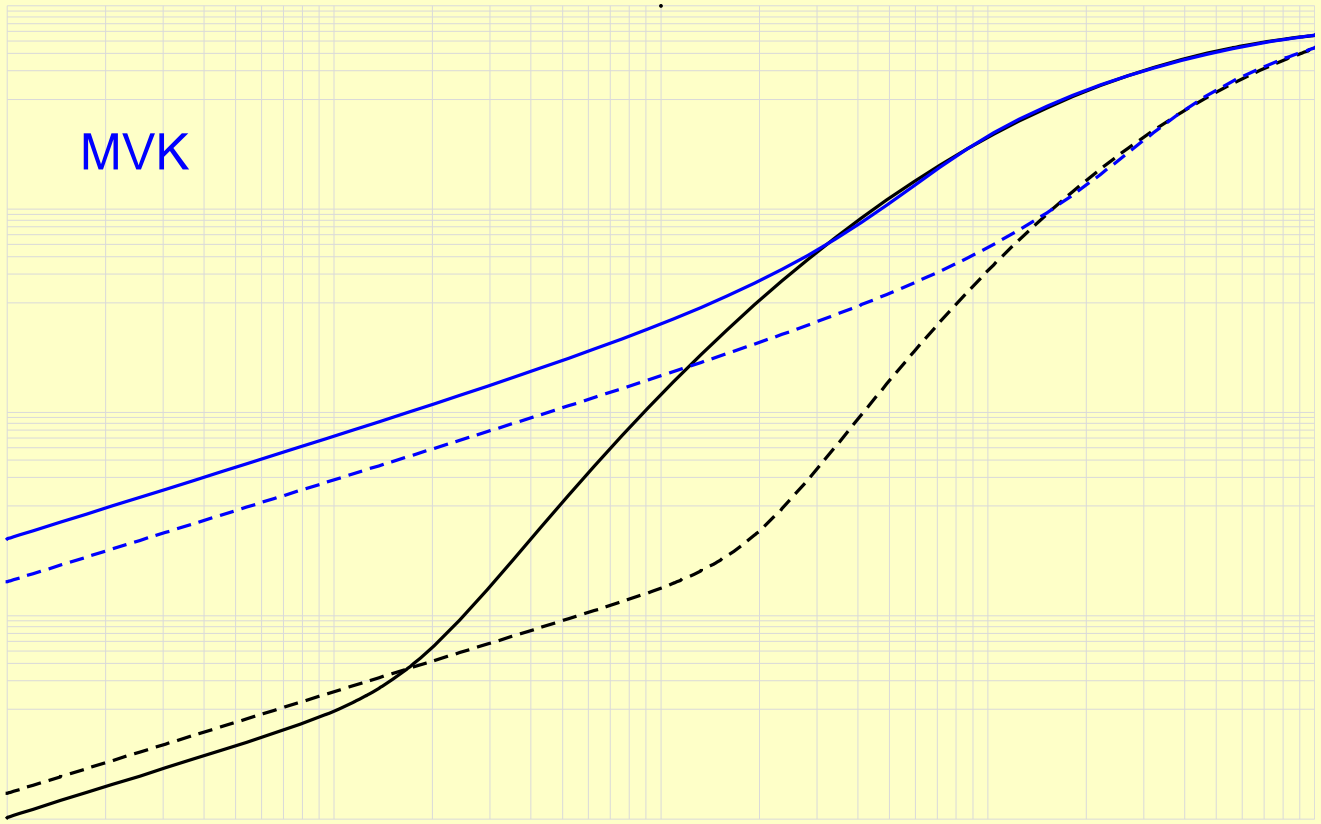
DAH and somatic-mutation/proliferation models can predict profoundly different dose-response relationships for mutagenic carcinogens

- ILLUSTRATION: Dibenzo[*a*,*l*]pyrene
- One of the most potent mutagenic chemicals identified in cigarette smoke
- MVK vs. DAH fits to cancer bioassay data from the ED_{001} study involving >40,000 trout administered one of 8 dietary doses (0 to 225 ppm) of DB[*a*,*l*]P for 4 weeks, then followed for 9 months

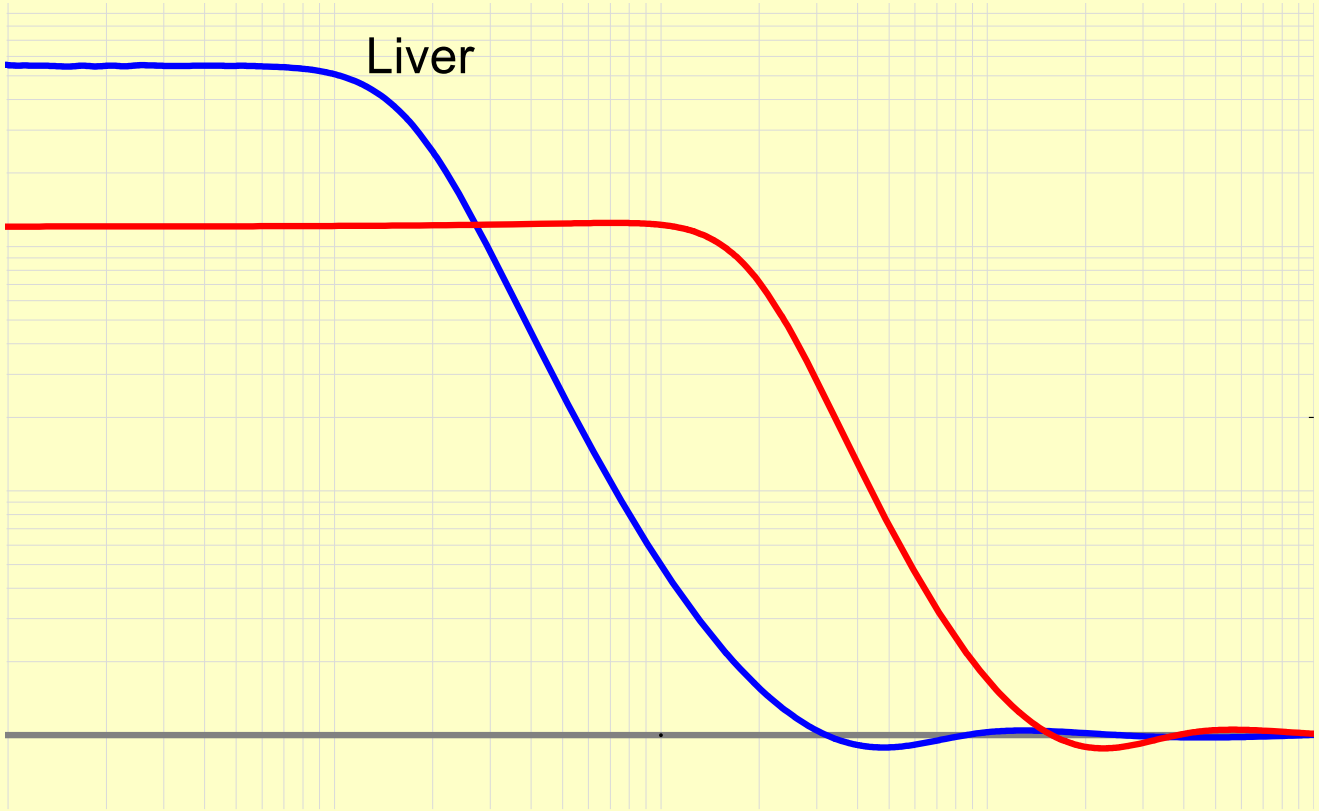
DAH and *non*-genotoxic MVK models fit the liver & stomach tumor incidence rates well



All these fits predict that risks increase in linear proportion to dose at the lowest levels of risk



However, at low doses (<0.25 ppm), MVK-based extrapolations of increased risk are far more conservative than DAH-based extrapolations



Conclusions

- The Dysregulated Adaptive Hyperplasia (DAH) theory of tumorigenesis
  - Combines key elements of other current theories
  - Implies separate, efficient pathways to benign vs. malignant tumors via mutation-induced failure to resolve P- vs. R-type AH
  - May predict profoundly nonlinear, hockey-stick-like low-dose dose-response behavior, even for mutagenic carcinogens
  - Only an improved mechanistic understanding of tumor biology—rather than any feasible refinement of dose-response data—can establish which model, DAH or MVK, is correct