

Indirect-acting carcinogens

Metabolic activation, DNA injury and cancer susceptibility

Chemical carcinogenesis • mechanisms and clinicopathologic correlations

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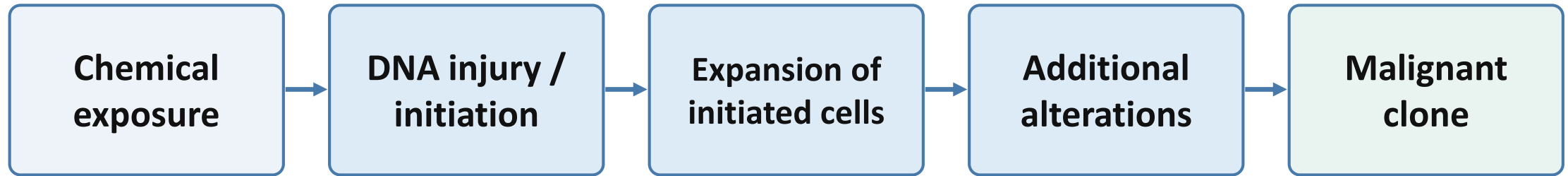
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Learning objectives

- Distinguish procarcinogens from ultimate carcinogens.
- Trace metabolic activation to DNA injury, initiation and clonal expansion.
- Recognize major exposure classes and their cancer associations.
- Interpret enzyme polymorphism and mutation signatures critically.

The central question: How does an initially inactive chemical become mutagenic?

Chemical carcinogenesis is a multistep process



A single exposure need not produce a fully malignant tumor.
Initiation and promotion are mechanistically different events.

Pott's observation linked soot to cancer

- Sir Percival Pott linked scrotal skin cancer in chimney sweeps to soot exposure.
- The association preceded modern identification of specific carcinogenic molecules.
- It illustrates how occupational observation can implicate environmental carcinogens.

Historical inference

Exposure pattern

**→ elevated cancer
occurrence**

→ etiologic hypothesis

Chemical initiators fall into two activation classes

Direct-acting

- Chemically active without metabolic conversion
- Includes several alkylating agents
- Can damage DNA directly

Indirect-acting

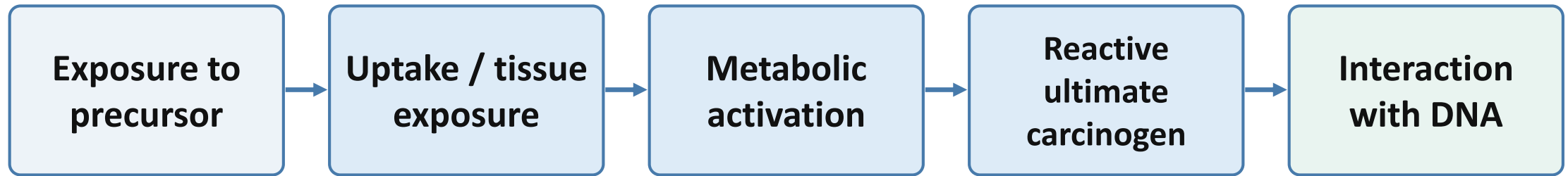
- Requires endogenous metabolic conversion
- Initial substance is a procarcinogen
- The reactive product is the ultimate carcinogen

Metabolic conversion defines indirect carcinogens

Procarcinogen $\neq$ ultimate carcinogen

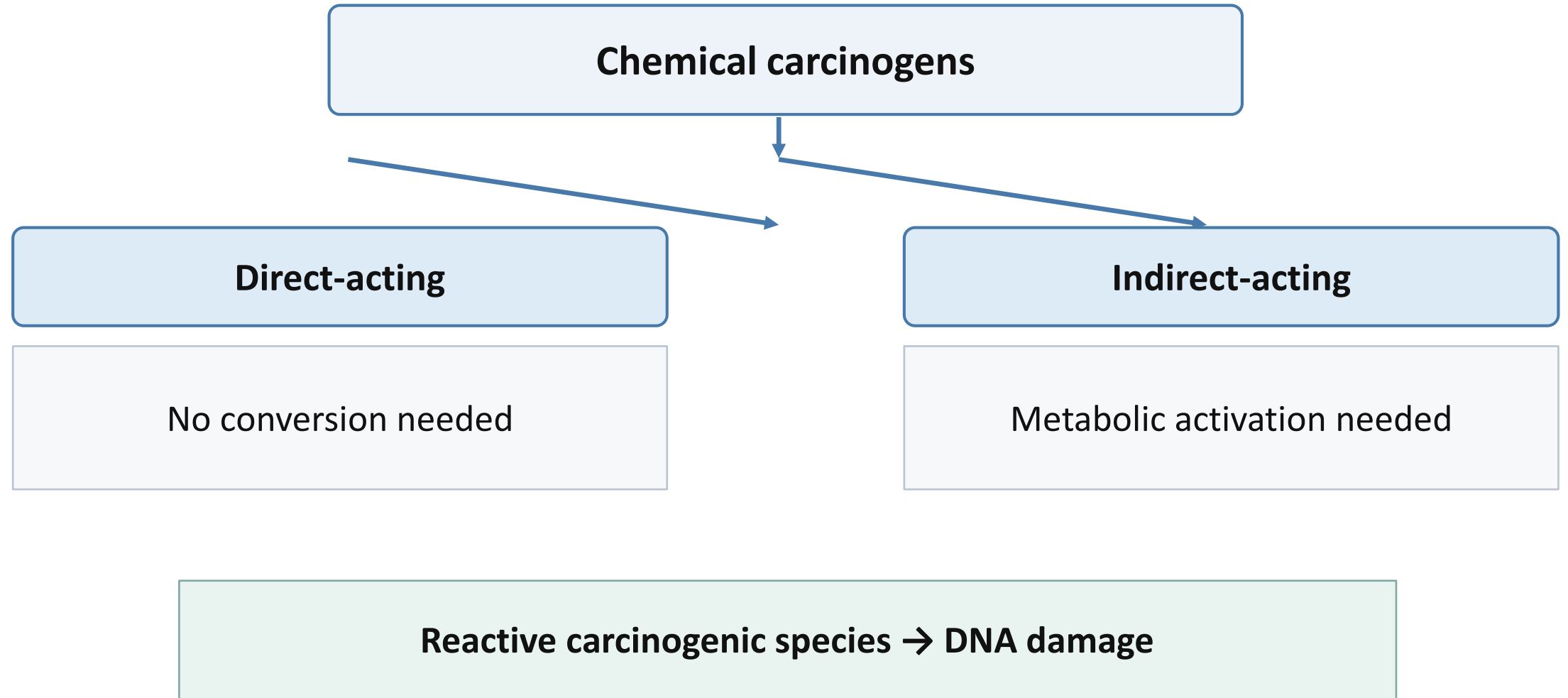
- Procarcinogen: Precursor that requires activation
- Ultimate carcinogen: Active product capable of initiating damage
- Conversion is commonly mediated by host metabolic enzymes

A procarcinogen becomes dangerous after activation



The active form—not merely the parent exposure—is pivotal to initiation.

Direct and indirect agents converge on DNA injury

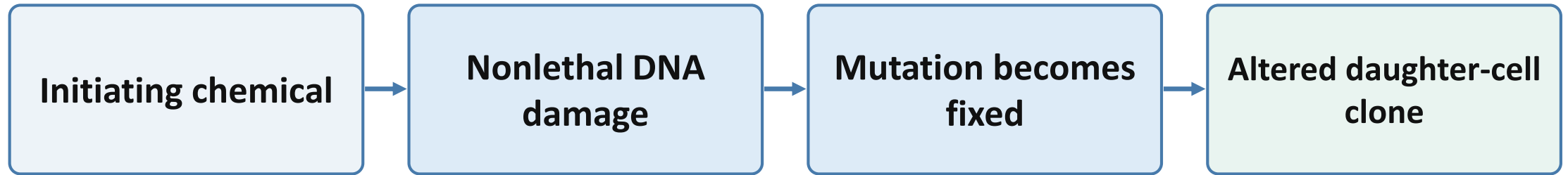


Not every exposed cell becomes malignant

- Carcinogens initiate through genetic injury; A malignant phenotype requires further alterations.
- DNA repair, persistence of mutation and subsequent proliferation influence outcome.
- The textbook separates initiation from the promotional expansion of an altered clone.

Exposure is the start of a sequence, not an automatic cancer diagnosis.

Initiation creates heritable genetic change



Initiation involves permanent genetic change, not simply temporary chemical exposure.

Electrophiles are the reactive initiating species

- Chemical initiators are reactive electrophiles: They contain electron-deficient atoms.
- Electrophiles interact with nucleophilic, electron-rich sites in cells.
- DNA is a critical molecular target because permanent mutations drive transformation.

Chemical interaction

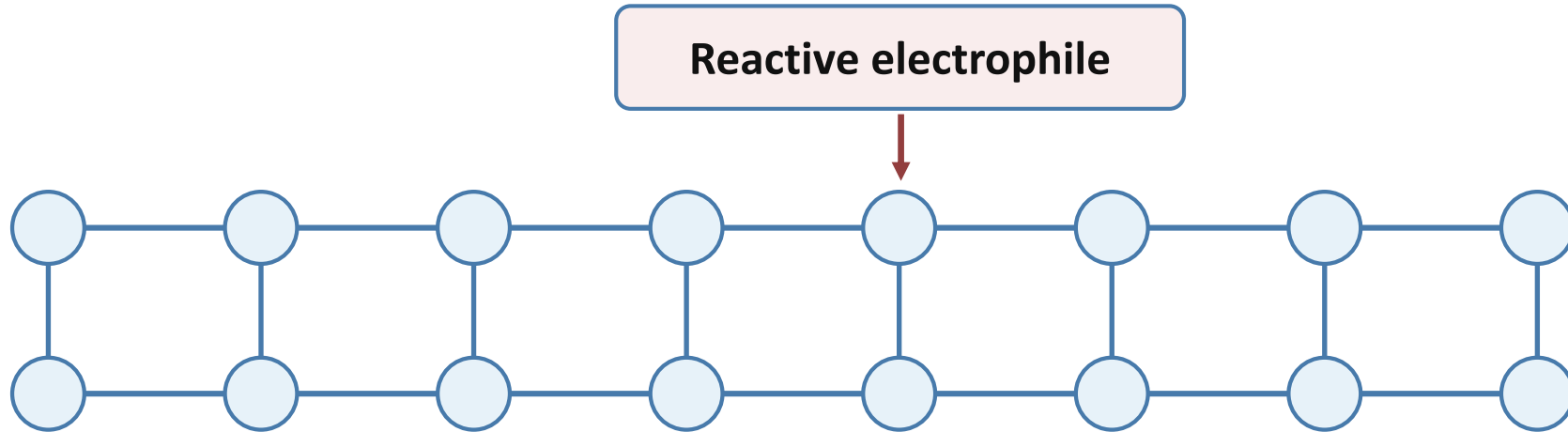
Electron-poor electrophile

↓ reaction

**Electron-rich cellular
target**

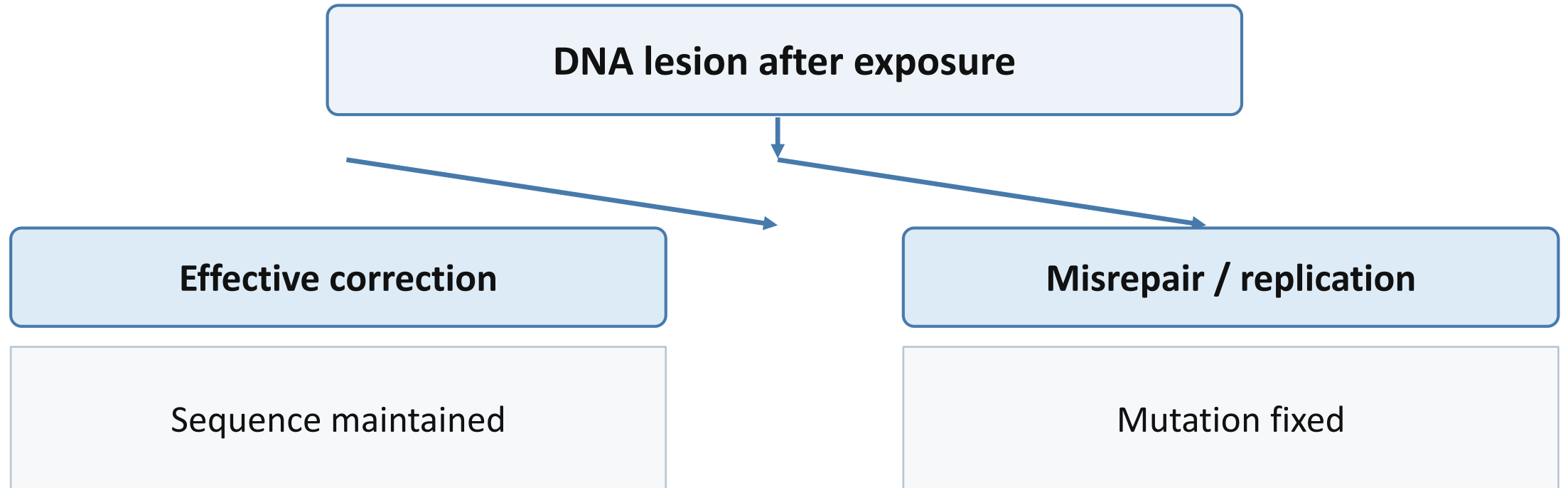
DNA provides nucleophilic molecular targets

Schematic: Reactive electrophile encounters DNA



- Reactive chemicals may form covalent lesions at susceptible DNA sites.
- The relevant carcinogenic effect is an altered DNA sequence after inaccurate repair or replication.
- A lesion is not equivalent to a permanently established mutation.

DNA injury has more than one outcome



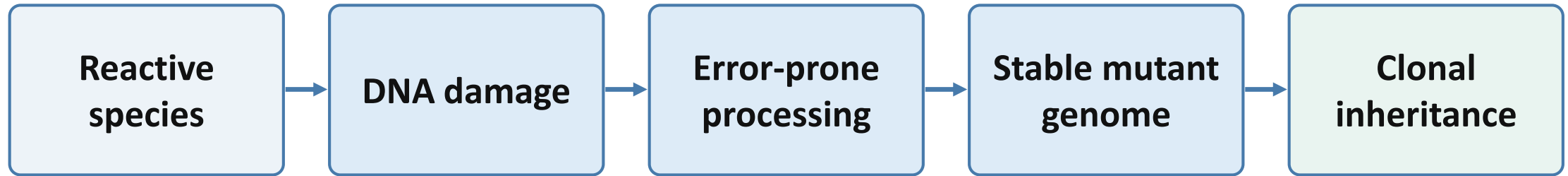
Only fixed mutations can be inherited by descendant cells

An initiated cell can appear viable

- Initiating damage is described as nonlethal in the classic model.
- The cell survives to transmit the altered DNA sequence to progeny.
- The resulting clone may remain clinically silent until further progression.

Initiation concerns molecular inheritance, not a recognizable tumor mass.

Mutation fixation makes initiation persistent



Removal of the external exposure does not undo an already fixed mutation.

Promotion expands initiated cells



Promotion is not synonymous with an independent DNA-mutating exposure.

Initiator and promoter play distinct roles

Feature	Initiating agent	Promoting influence
Primary action	Induces permanent genetic change	Stimulates proliferation
DNA mutation	Central to initiation	Not required for its defining role
Alone in classic model	Can initiate altered cells	Not tumorigenic alone
Effect on clone	Creates altered founder	Expands initiated descendants

The sequence of events explains experimental tumors

- Classic mouse-skin studies distinguished initiation from promotion.
- Promoters produce tumors by stimulating already initiated cells.
- Clonal expansion raises opportunity for additional genetic alterations.

Conceptual order

Initiate

→ Promote

→ Progress

Promoters can be physiologic or inflammatory

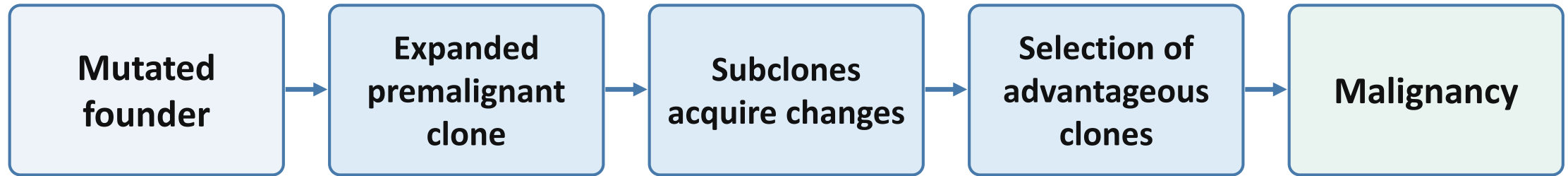
Hormonal proliferation

- Unopposed estrogenic stimulation
- Endometrial and breast tissue
- Illustrates growth-promoting context

Chronic tissue repair

- Persistent inflammation or fibrosis
- Examples include chronic hepatitis and inflammatory bowel disease
- Repeated cycles of injury and repair

A cancer needs cumulative alterations



The multistep model explains the interval between exposure and cancer emergence.

Major procarcinogen classes are chemically diverse

Class in Robbins	Illustrative agents	Common teaching context
Polycyclic / heterocyclic aromatic hydrocarbons	Benzo[a]pyrene; benz[a]anthracene	Combustion-related exposure
Aromatic amines, amides, azo dyes	2-naphthylamine; benzidine	Historical occupational exposure
Plant / microbial products	Aflatoxin B ₁ ; safrole; cycasin	Natural-source exposures
Other listed agents	Nitrosamines; vinyl chloride; PCBs	Environment / industry / food chemistry

Polycyclic aromatic hydrocarbons are potent procarcinogens

- Polycyclic hydrocarbons among the most potent indirect chemical carcinogens.
- They occur in fossil-fuel products and combustion-derived material.
- A prominent teaching example is benzo[a]pyrene.

Chemical class links sources, metabolic activation and DNA damage.

Soot and fossil fuels are exposure reservoirs

- Polycyclic hydrocarbons are present in fossil fuels.
- Soot provided a historically important exposure signal in chimney sweeps.
- The carcinogenic effect depends on constituent chemicals, not the descriptive term “soot” alone.

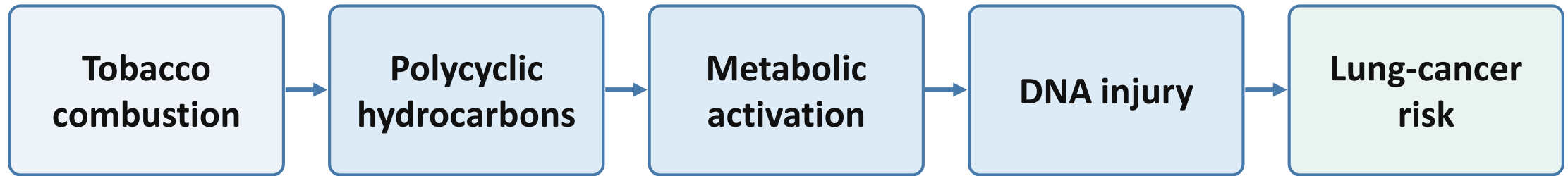
Exposure pathway

Fuel / carbon material

→ contaminated mixture

→ human contact

Tobacco combustion produces carcinogenic hydrocarbons



Benzo[a]pyrene is one example of a smoke-associated chemical carcinogen.

Benzo[a]pyrene is a model indirect carcinogen

- Produced during high-temperature combustion, including tobacco burning.
- Belongs to the polycyclic aromatic hydrocarbon class.
- Requires conversion by metabolic enzymes to a reactive carcinogenic form.

Model teaching chain

Benzo[a]pyrene

**→ P450-mediated
activation**

**→ DNA-reactive
metabolites**

Combustion exposures span several environments

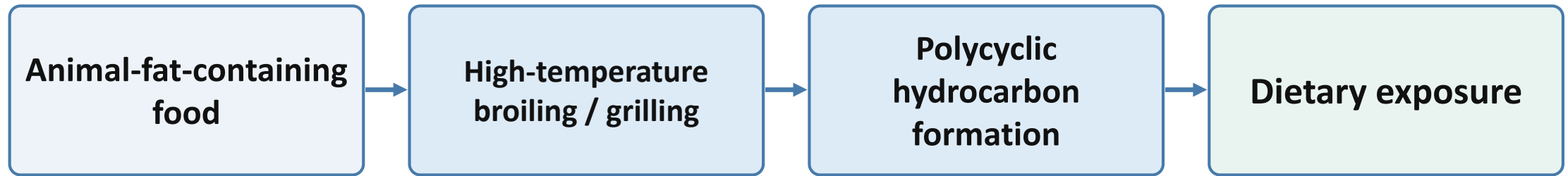
Inhalational settings

- Cigarette smoke
- Soot and combustion products
- Historical occupational contact

Dietary settings

- Broiled or grilled meat
- Smoked meat
- Smoked fish

High-temperature cooking can generate hydrocarbons



Robbins also identifies smoked meats and fish as sources.

Exposure source is not equal to dose or individual risk

- Identifying a source establishes a possible exposure pathway.
- Actual carcinogenic risk also depends on the amount and pattern of exposure.
- Metabolic activation, inactivation and later biologic events modify outcome.

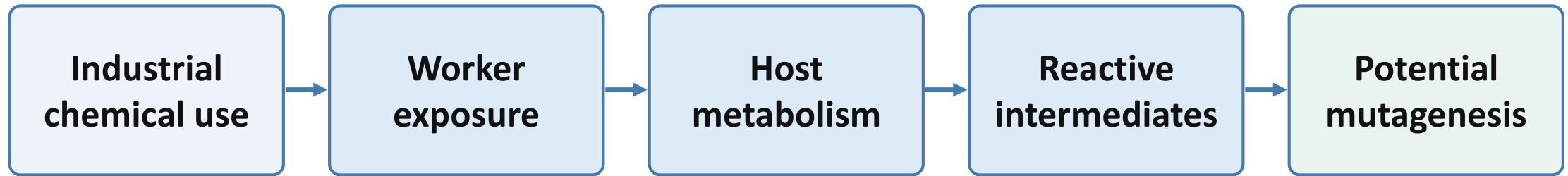
Do not equate the presence of a named carcinogen with inevitable malignancy.

Aromatic amines and azo dyes form another major class

- These chemicals are identified as indirect-acting carcinogens.
- Historically used extensively in aniline-dye and rubber industries.
- Examples listed include 2-naphthylamine, benzidine and dimethylaminoazobenzene.

An industrial exposure history can provide etiologic clues.

Historical industries illustrate occupational risk



The pathway is conceptual; Individual agents and occupational conditions vary.

Representative aromatic compounds are not interchangeable

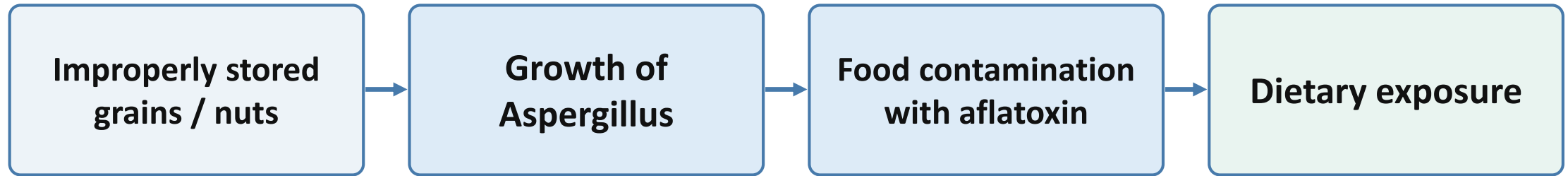
Agent named by Robbins	Table 7.10 category	Important interpretation
2-Naphthylamine	Aromatic amine	Specified chemical; do not substitute a generic dye
Benzidine	Aromatic amine	Distinct agent within the same broad class
2-Acetylaminofluorene	Aromatic amide	Listed among activation-dependent compounds
Dimethylaminoazobenzene	Azo dye	Also called “butter yellow”

Aflatoxin B₁ is a natural-source carcinogen

- Aflatoxin B₁ as a carcinogen from certain strains of *Aspergillus*.
- This illustrates that “natural” exposure is not synonymous with biologic safety.
- The clinically relevant tumor association in the text is hepatocellular carcinoma.

The example also demonstrates an exposure-linked mutation hotspot.

Improper grain and nut storage permits fungal growth



The exposure pathway precedes the molecular events in hepatic cells.

Aflatoxin exposure has a geographic association

- Strong correlation between dietary aflatoxin contamination and hepatocellular carcinoma in parts of Africa and Asia.
- This is an epidemiologic association described.
- Regional patterns should not be misconstrued as uniform individual exposure.

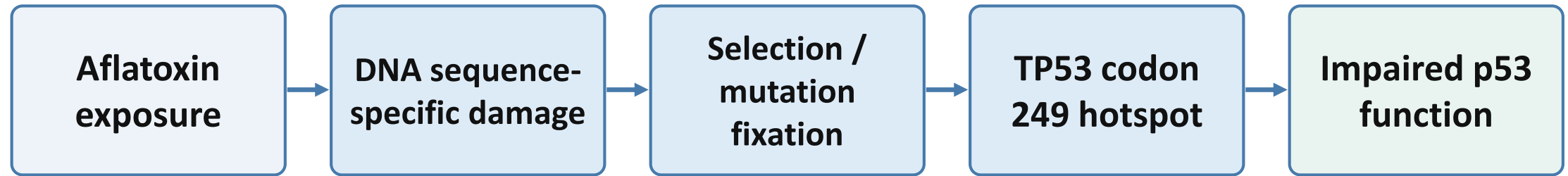
Observed relationship

Contaminated food

→ dietary exposure

**→ associated HCC
incidence**

Aflatoxin-linked cancers can show TP53 hotspots



This is an association and mechanistic illustration, not a universal diagnostic criterion.

TP53 codon 249 exemplifies a point mutation

G:C → T:A transversion

- Codon 249 is a characteristic hotspot in aflatoxin-associated HCC.
- The resulting amino-acid substitution is Arg → Ser (R249S).
- The variant compromises p53 tumor-suppressor function.

A hotspot is enrichment, not exclusivity

What hotspot means

- A recurrently affected genomic position
- Preferential chemical / sequence interaction
- An enriched lesion in exposed tumors

What it does not mean

- All exposed people carry the mutation
- Every HCC has the substitution
- A single mutation proves the exposure history

The mutation pattern differs by exposure context

Setting described in Robbins	TP53 codon 249 observation
Aflatoxin-associated HCC	Characteristic hotspot reported
Liver tumors from areas without aflatoxin contamination	TP53 mutations less frequent; few at codon 249
Interpretive boundary	An association, not a standalone clinical test

Most chemical initiating agents target DNA

- Mutagenicity follows from the fact that malignant transformation depends on genetic alterations.
- DNA is the major target of chemical initiators.
- Initiation does not depend on one exclusive carcinogen-specific cancer gene.

Damage can occur broadly; Selection determines which mutant cells expand.

A DNA lesion and a driver mutation are different

DNA lesion / mutation

- Chemical interaction may damage many sites
- Many mutations have little selective effect
- Mutational patterns reflect exposure chemistry

Selected driver alteration

- Provides a growth or survival advantage
- May involve an oncogene or tumor suppressor
- Can contribute to stepwise progression

Mutations are distributed but not chemically uniform

Reactive chemical interacts with DNA

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graph TD; A[Reactive chemical interacts with DNA] --> B[Numerous genomic sites]; A --> C[Preferred bases / sequences]; B --> D[Mutations may be widespread]; C --> E[Hotspots and characteristic substitutions]; D --> F[Selection expands clones with biologic advantage]; E --> F;
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Numerous genomic sites

Mutations may be widespread

Preferred bases / sequences

Hotspots and characteristic substitutions

Selection expands clones with biologic advantage

RAS and TP53 illustrate selection after mutation

- Carcinogen-induced changes are not restricted to one specific cancer gene.
- Cells that acquire advantageous alterations may clonally expand.
- RAS and TP53 as illustrative oncogene and tumor-suppressor targets.

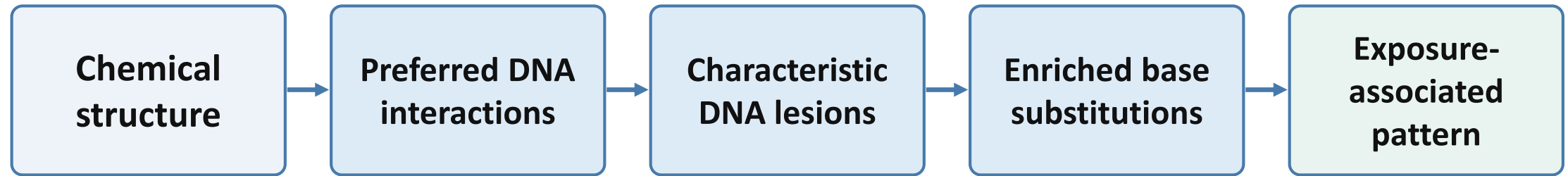
Evolutionary logic

Mutation occurs

→ selective advantage

→ expansion of clone

DNA chemistry can bias substitution patterns



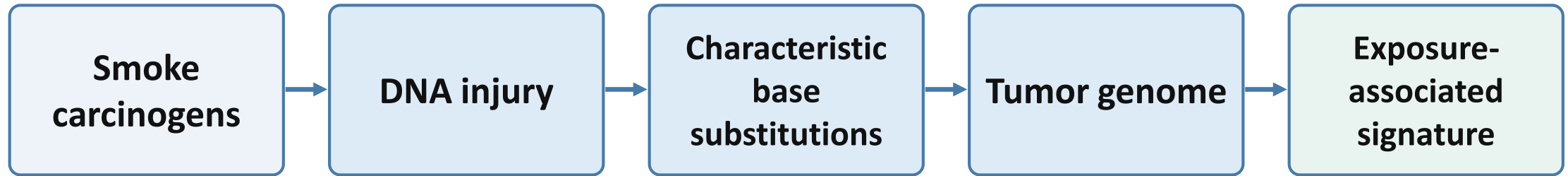
Mutations need not occur at uniformly random DNA positions.

Smoking-associated lung cancers carry greater mutational burden

≈ 10 -fold greater average mutational burden

- Smoking-associated lung cancers with lung cancers in nonsmokers.
- The excess includes substitutions associated with tobacco-smoke carcinogens.
- This is a tumor-group average reported in the supplied text, not a patient-level cutoff.

Tobacco produces a recognizable substitution bias



Mutational burden and mutational spectrum provide different kinds of information.

Mutational burden and signature answer different questions

Genomic observation	Interpretive question	Robbins example
Mutational burden	How many alterations occur?	Higher average burden in smoking-related lung cancer
Substitution spectrum	What kinds of nucleotide changes?	Smoking-associated base substitutions
Hotspot	Which site recurs?	TP53 codon 249 in aflatoxin-associated HCC

A mutational signature is a pattern, not a diagnosis

- Cancer genome sequencing identifies characteristic combinations of mutations.
- Some patterns can be associated with specific carcinogenic exposures.
- Pattern recognition alone does not establish every individual exposure or cause.

Use genomic findings alongside epidemiology and exposure history.

Not every mutational signature has a known cause

- Dozens of mutational signatures have been described.
- Some signatures reflect exposure to chemotherapeutic agents.
- Many remain unexplained; A proposed causal exposure requires further evidence.

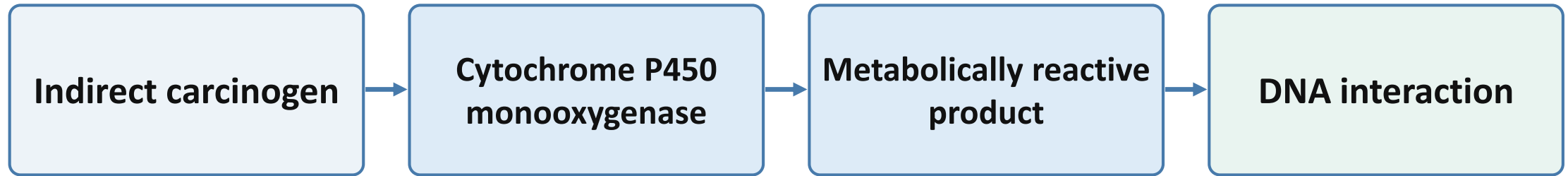
Scientific interpretation

Recognized pattern

≠

Proven single exposure

Cytochrome P450 activates many procarcinogens



Most indirect carcinogens are described as requiring P450-dependent metabolism.

Metabolic enzymes are endogenous host factors

- The activating enzymes are produced by the exposed individual.
- Their genes influence how efficiently a precursor is converted.
- Thus, the same external exposure can have biologically different consequences.

Susceptibility partly reflects the interaction of exposure and metabolism.

P450 enzyme genes are polymorphic

- Different allelic variants can encode different enzyme activity or inducibility.
- These inherited differences are identified as one determinant of carcinogenesis susceptibility.
- A gene variant modifies risk; It does not independently establish cancer.

Risk modifiers

Inherited enzyme variant

→ activation capacity

**→ reactive metabolite
load**

Inducibility and basal activity are not identical

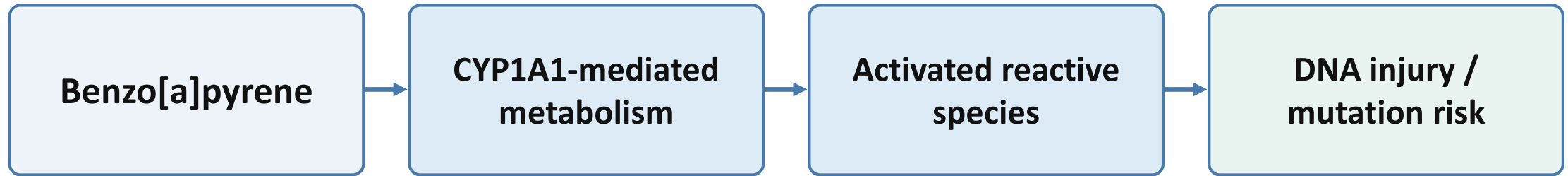
Enzyme activity

- Catalytic behavior at a given state
- Affects conversion of procarcinogen
- May vary among individuals

Enzyme inducibility

- Capacity to increase enzyme expression / activity
- Can alter conversion following exposure
- Also subject to genetic variation

CYP1A1 illustrates activation of benzo[a]pyrene



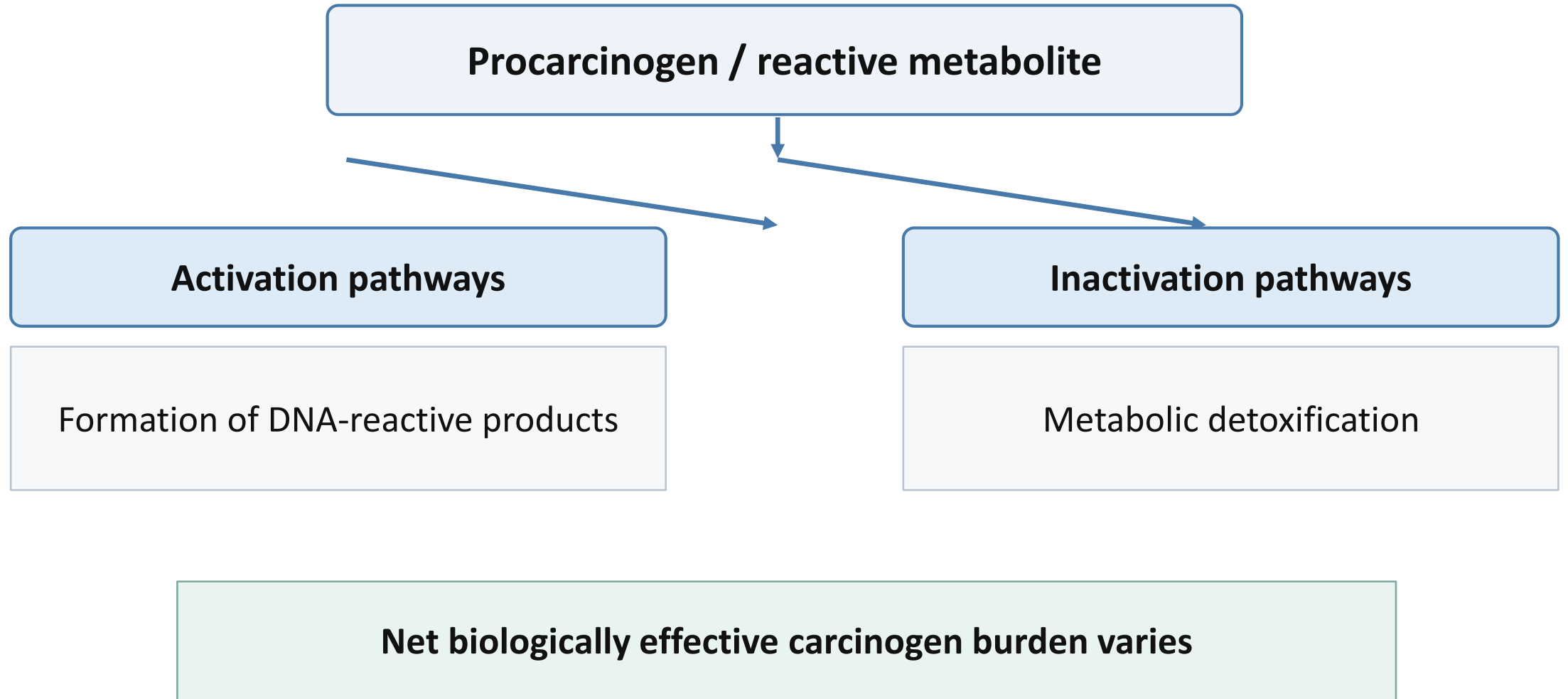
This is the specific P450 example used by Robbins.

A high-inducibility CYP1A1 variant

Inherited CYP1A1 variability affects activation potential

- Textbook example: ~10% of people of Northern European descent have a highly inducible form.
- Up to sevenfold higher lung-cancer risk in smokers with this genotype versus smokers without it.
- These are source-reported population associations, not universal predictive estimates.

Activation and detoxification are competing fates



Variation in detoxification can modify risk

- Metabolism can inactivate a precursor or derivative, not merely activate it.
- Differences in these pathways can change the amount of damaging metabolite present.
- Risk depends on the balance between generation and removal of active species.

Two-way metabolism

Activation ↑ → injury
potential ↑

Detoxification ↑ →
burden ↓

Cancer susceptibility cannot be read from one gene

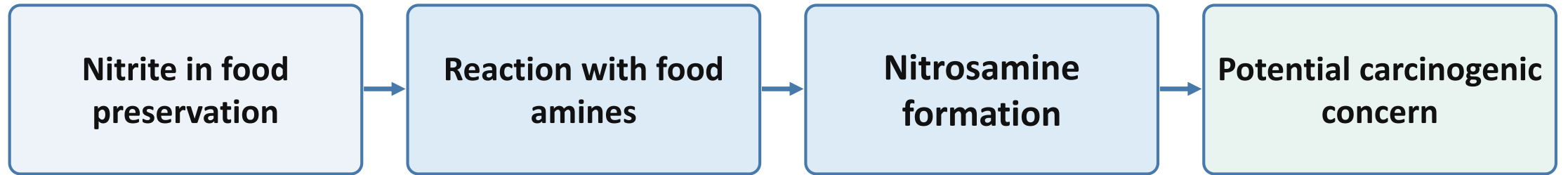
- Chemical exposure, activating enzymes and detoxifying pathways all matter.
- DNA repair and the proliferation of initiated cells are additional steps in the model.
- Genetic predisposition is probabilistic; The source does not establish a clinical predictive test.

Avoid deterministic language when interpreting metabolic polymorphisms.

Other environmental agents appear' table

Source grouping	Selected entries listed
Other occupational / environmental	Vinyl chloride; nickel; chromium
Additional chemical concerns	Pesticides; polychlorinated biphenyls
Natural products	Griseofulvin; cycasin; safrole; betel nuts
Food-related chemistry	Nitrosamines / related amides

Food nitrites can participate in nitrosamine formation



Nitrosamines formed this way as suspected carcinogens; Do not imply quantified dietary risk.

Exposure history organizes clinicopathologic reasoning

Exposure inquiry

- Tobacco and combustion products
- Occupation / industrial chemicals
- Food storage and dietary contamination

Biologic interpretation

- Possible metabolic activation
- Potential DNA damage signature
- Context of tumor type and host factors

Case: Cigarette-smoke exposure

A patient with lung cancer has a prolonged smoking history. A tumor sequencing report shows many base substitutions.

Discussion prompts

- What feature of smoke connects exposure to DNA damage?
- Why does the mutation spectrum complement—but not replace—the exposure history?

High-temperature combustion generates indirect chemical carcinogens, including benzo[a]pyrene. Activation and characteristic DNA substitutions may leave an exposure-associated signature.

Case: Grain storage and hepatocellular carcinoma

A region with frequent food contamination from improperly stored grains has increased hepatocellular carcinoma incidence.

Discussion prompts

- Which natural-source carcinogen is?
- Which tumor-suppressor hotspot is classically associated?

Aflatoxin B₁ produced by certain *Aspergillus* strains. TP53 codon 249 G:C → T:A transversion with R249S substitution.

Case: Genetically variable metabolism

Two smokers report comparable tobacco exposure but differ in genetically determined P450 inducibility.

Discussion prompts

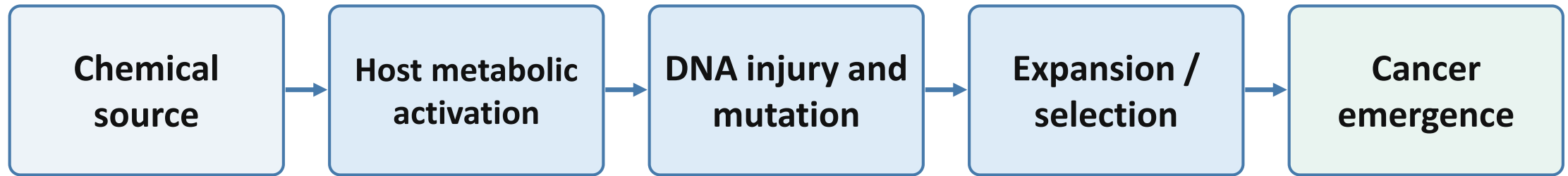
- Can exposure be translated into identical amounts of ultimate carcinogen?
- What additional metabolic fate must be considered?

No. Activation rates may vary with enzyme polymorphisms and inducibility. Inactivation / detoxification pathways also influence net metabolite burden.

Compare three representative examples

Example	Exposure setting	Molecular teaching point
Benzo[a]pyrene	Tobacco / soot / combustion	P450 bioactivation; tobacco-linked mutation patterns
Aflatoxin B ₁	Contaminated food	Association with TP53 codon 249 hotspot
Aromatic amines / azo dyes	Historical dye and rubber industry	Occupational procarcinogens needing activation

A defensible causal chain has several links



Each link should be supported separately when interpreting a real patient or population.

Five distinctions prevent common errors

- Procarcinogen is not the same as its ultimate carcinogen.
- Chemical initiation is not the same as proliferative promotion.
- DNA lesions are not identical to permanently fixed mutations.
- Mutation signatures are not always a unique proof of exposure.
- Metabolic susceptibility does not make malignancy inevitable.

Mechanism, exposure, genome and clonal evolution must be interpreted together.

Primary reading

- Robbins, Cotran & Kumar: Pathologic Basis of Disease.
- 11th edition (2026).
- Sections: Chemical Carcinogenesis; Indirect-Acting Carcinogens; Molecular Targets of Chemical Carcinogens.
- Conceptual figures and teaching diagrams in this lecture are newly constructed, not reproduced textbook images.