Cadmium acts as a mutagen by inhibiting mismatch repair.
(Genotoxicity caused by inhibition of a mutation avoidance system)

Dmitry A. Gordenin
National Institute of Environmental Health Sciences (NIEHS)

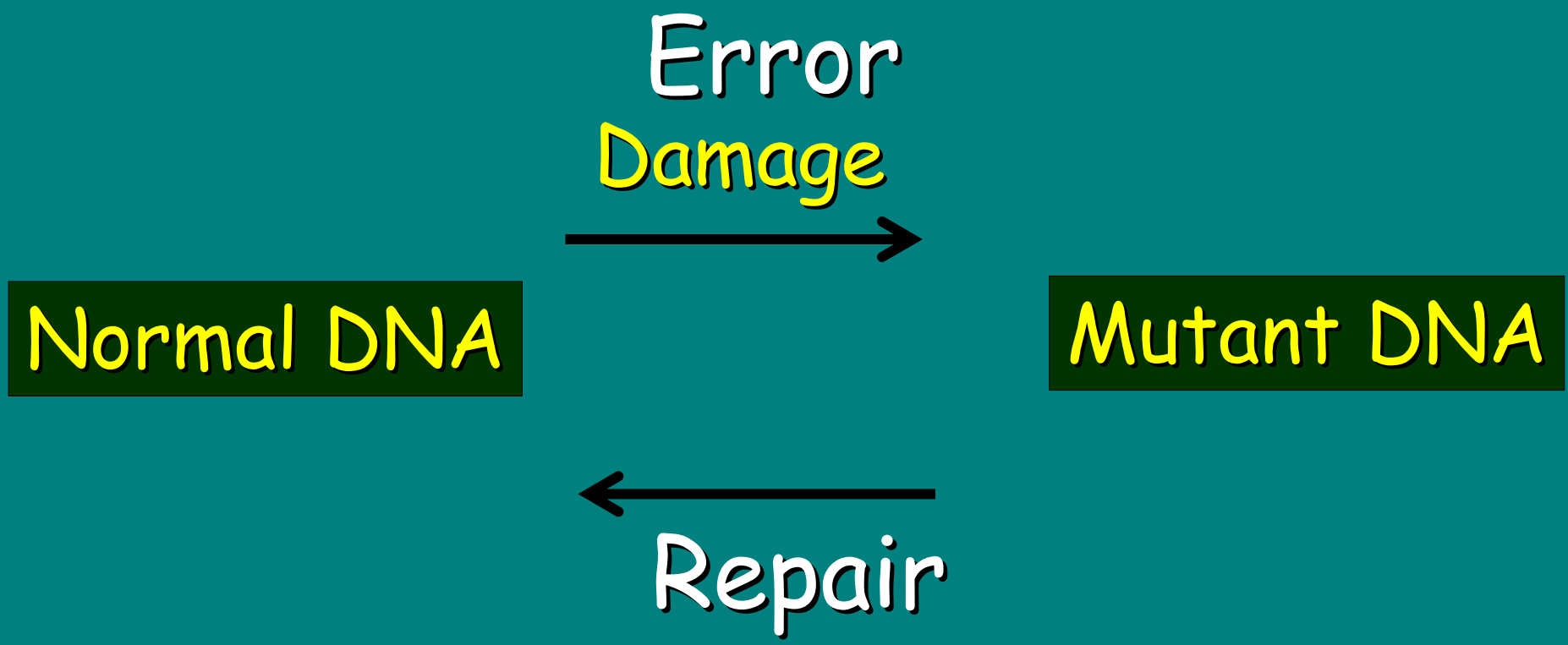
COLLABORATORS (all from NIEHS):

Yong Hwan (Yohan) Jin
Hanan Al-Refai
Michael Resnick

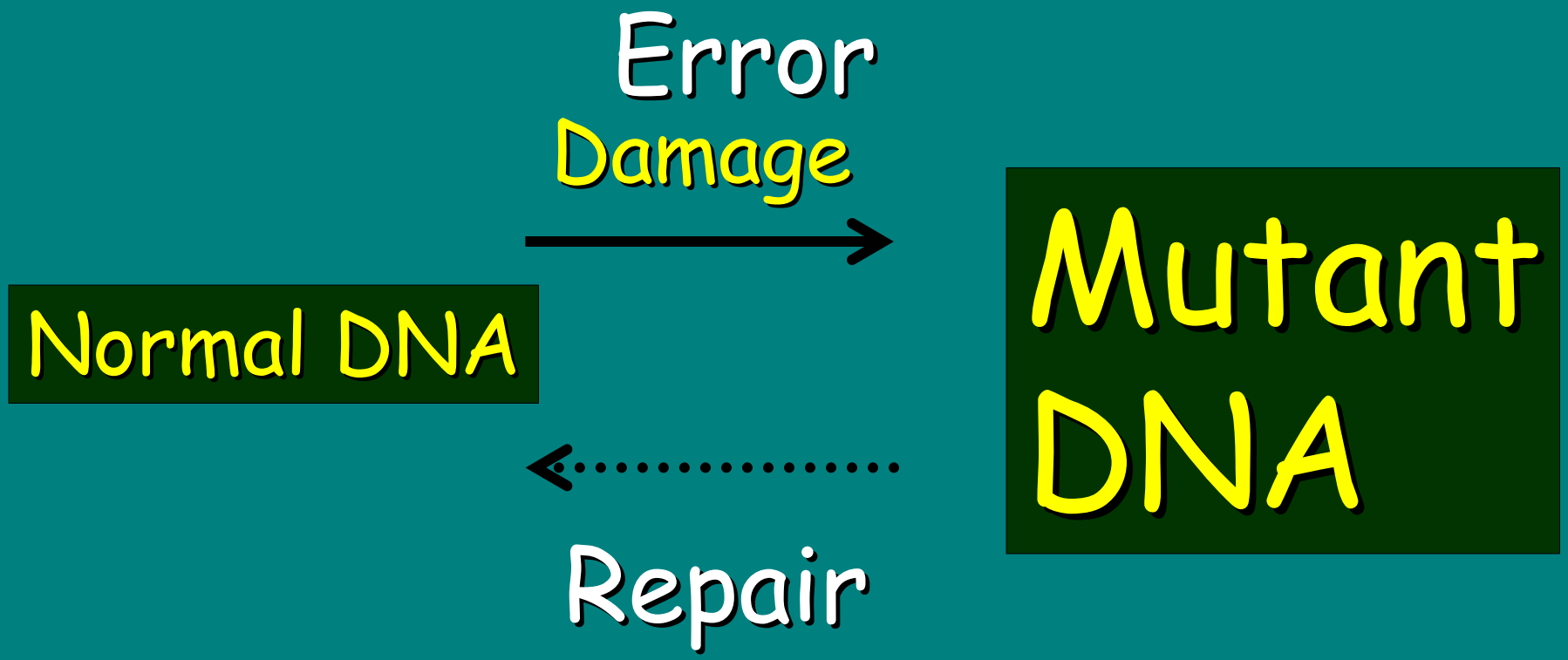
Alan Clark
Tom Kunkel

Rob Slebos
Jack Taylor

Environmental factors can cause genome instability



Can hyper-mutability be caused by environmental factor inhibiting repair?



High Risks of Genome Instability

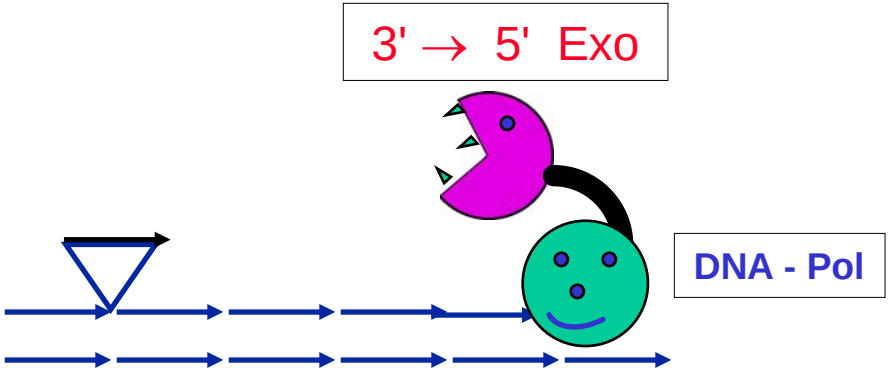
Combination of MMR and proofreading defects cause catastrophic mutability

(Morrison & Sugino; Schaaper)

- lethality (error catastrophe) in haploids

- synergy between mutators

At-Risk Motifs (ARMs) -- long homonucleotide runs



Long homonucleotide runs are hyper-mutable in MMR-deficient cells

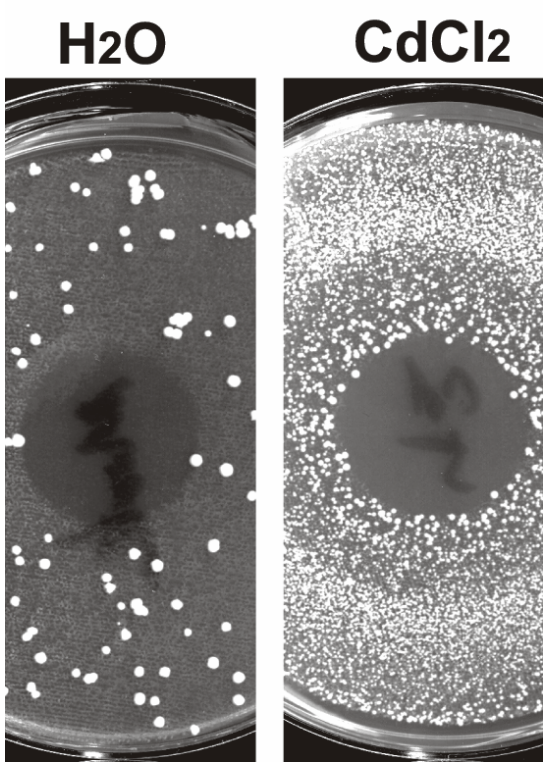
Long homonucleotide runs are hyper-mutable in MMR-deficient yeast

Homonucleotide run	Mutation rate ($x10^{-9}$)		*msh2* mutator
A14 (-1 nt)	164	1,600,000	x 9,756
A5 (+1 nt)	1	37	x 34
A8 (+1 nt)	10	3,440	x 344
A12 (+1 nt)	190	84,000	x 444

Mutation reporter - frameshifts in *lys2-A14*

- At-risk motif hyper-mutable in MMR-mutants
- Allows to detect as little as 0.1% unrepaired mismatches

Cadmium is hyper-mutagenic in a yeast long homonucleotide run (*lys2-A14*)



1x >>100x

Cadmium (Cd^{++}) in nature (IARC, Vol. 58, 1993)

112 μg=1μmole

- **Natural occurrence:** 100-500 μg/kg of the Earth's crust (mainly associated with zinc)
- **Air:** 0.05-0.5 μg/m^3; occupational - mining, battery, paint, metal industries.
- **Water:** <0.005 μg/L - 405 μg/L
- **Soil and plants:** <1,000 μg/kg - 800,000 μg/kg
- **Cigarette smoke:** A smoker can accumulate 500 μg/year.
- **Food:** 10μg/day to 500 μg/day
- **Animal and human tissues:** Liver, kidney, prostate (0.1-500 mg/kg).

(The half-life of cadmium in human kidneys is around 10-20 years.)

Cadmium carcinogenicity and genotoxicity (IARC, Vol. 58, 1993; NTP 10th Report on Carcinogens, 2002)

Carcinogenicity:

- lung cancer and prostate cancer (limited evidence) in humans
- lung, testicular, adrenal, liver, prostate tumors as well as lymphomas in experimental animals

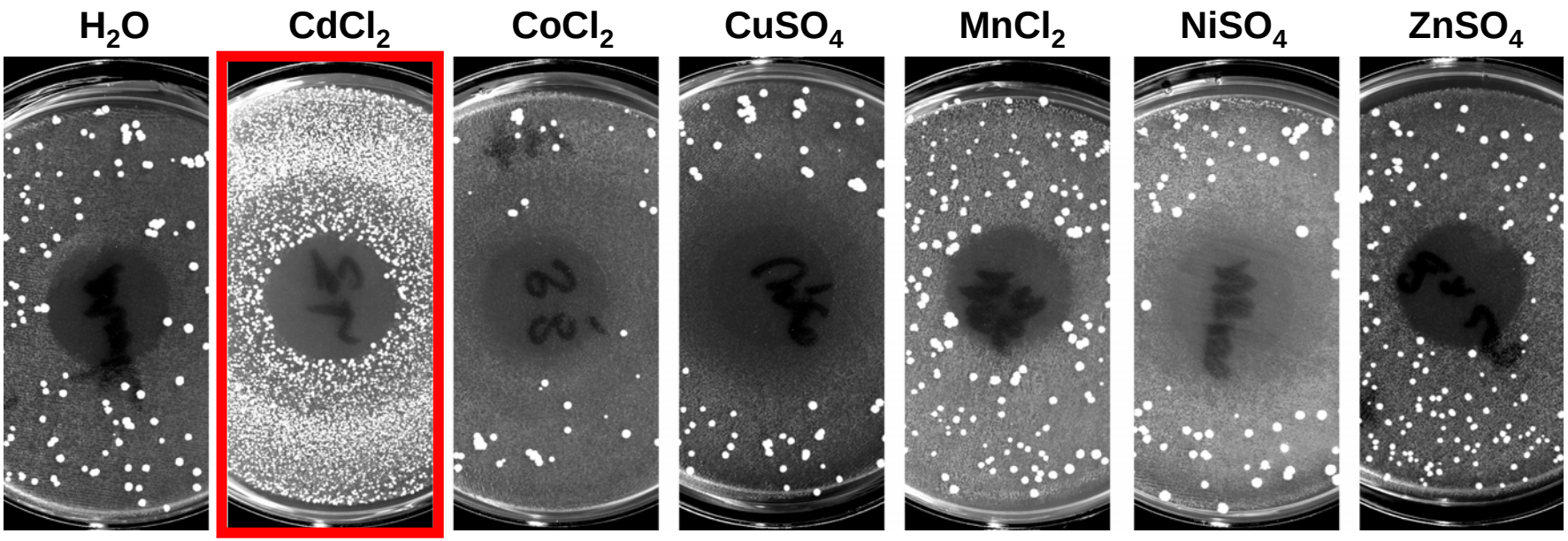
Genotoxicity (mostly from acute short-term treatment):

- chromosomal aberrations in lymphocytes of exposed workers
- chromosomal aberrations and strand breaks in cultured mammalian cells
- gene mutations in cultured mammalian cells (*hprt, gpt*)
- intra-chromosomal recombination in yeast

Multiple Potential Mechanisms of Metal Genotoxicity

- inhibiting repair
- suppressing fidelity
- oxidative damage to DNA
- oxidative damage to proteins
- complexes with DNA
- competing with "physiological" metals for metalothioneins

Cadmium caused hyper-mutability in yeast, unlike other ions tested



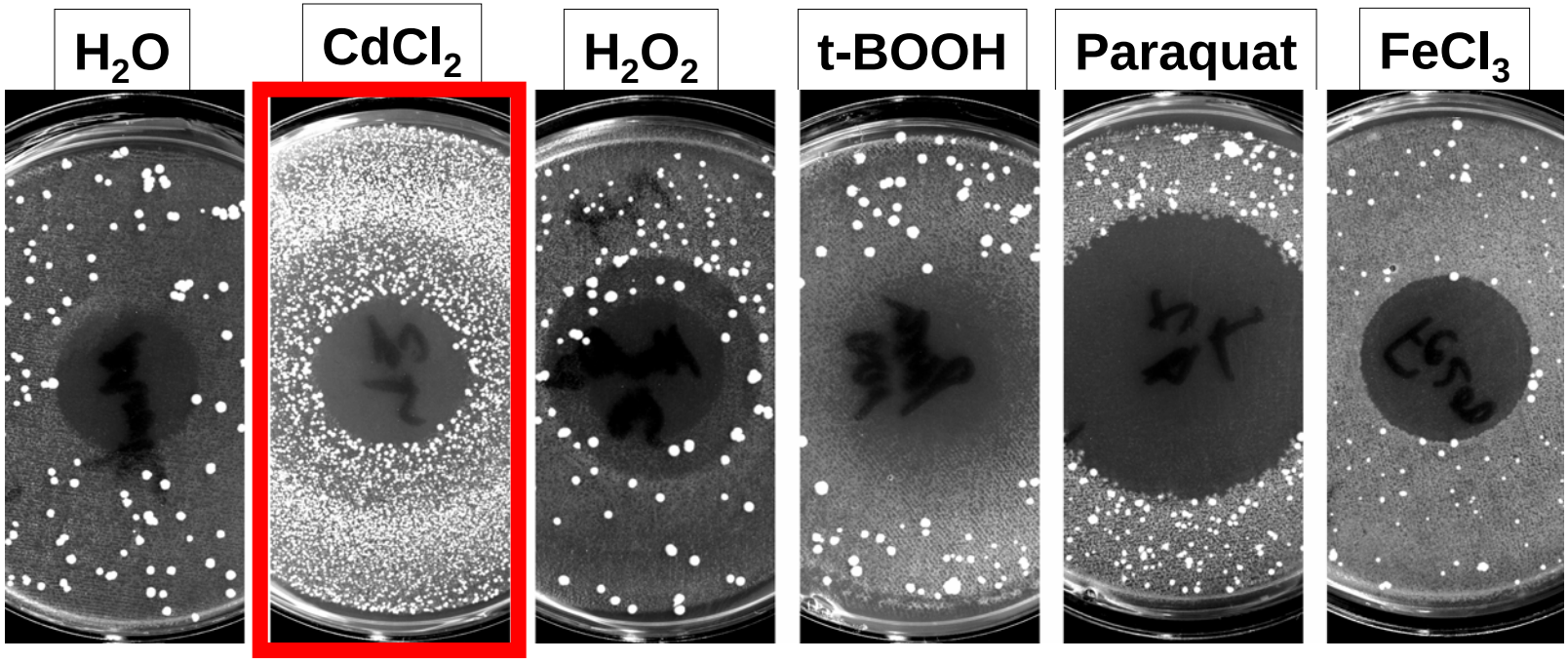
Mutation reporter - frameshifts in *lys2-A14*

At-risk motif hyper-mutable in MMR-mutants

Multiple Potential Mechanisms of Cadmium Genotoxicity

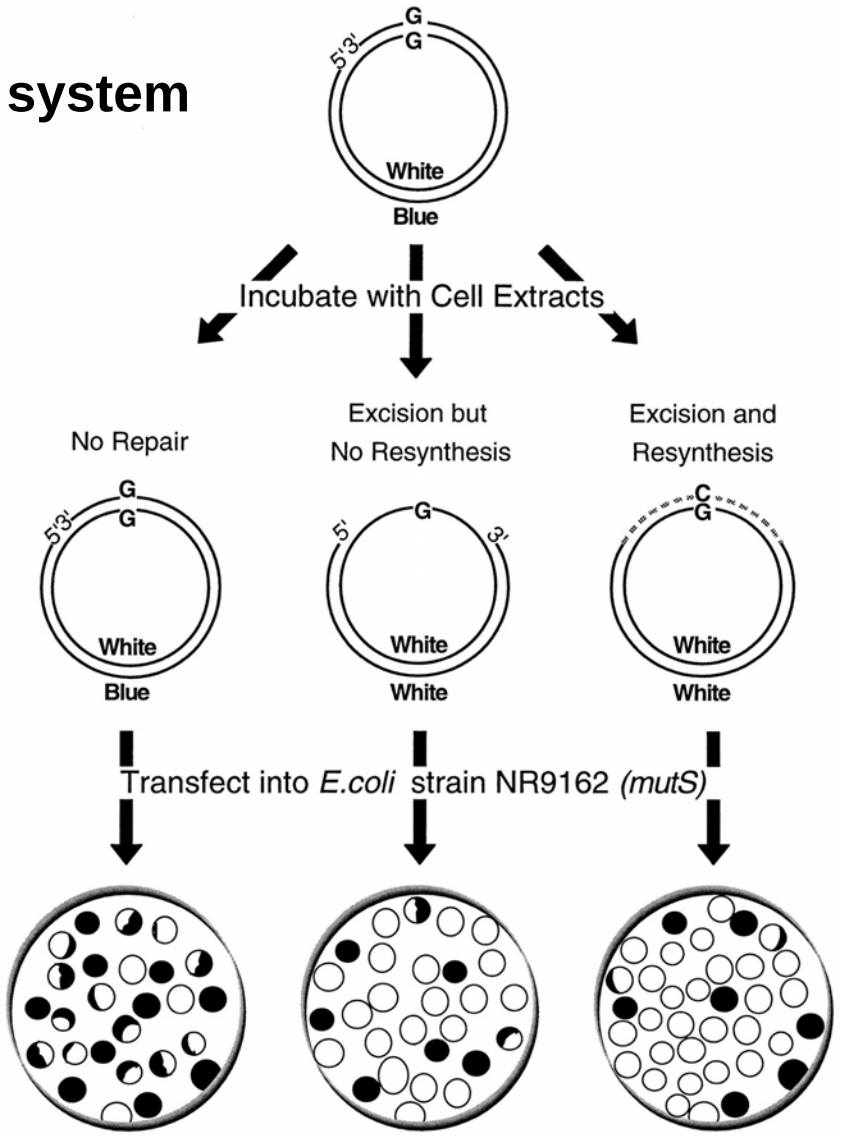
- inhibiting repair
- suppressing fidelity
- oxidative damage to DNA
- oxidative damage to proteins
- complexes with DNA
- competing with "physiological" metals for metalothioneins

Hyper-mutability is not due to general oxidative damage

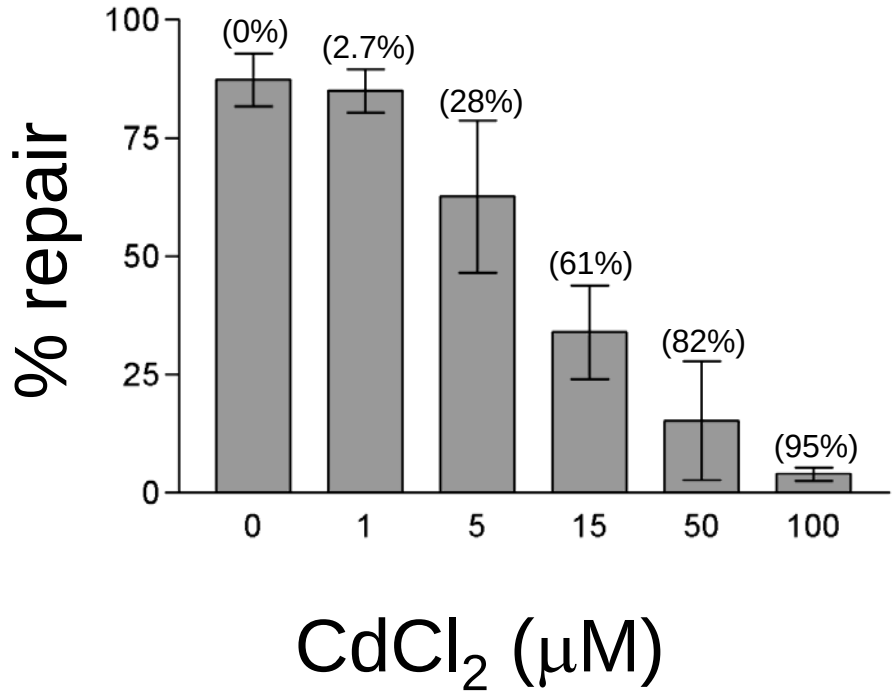


In vitro MMR assay system

(Umar et al., Cell 1996)

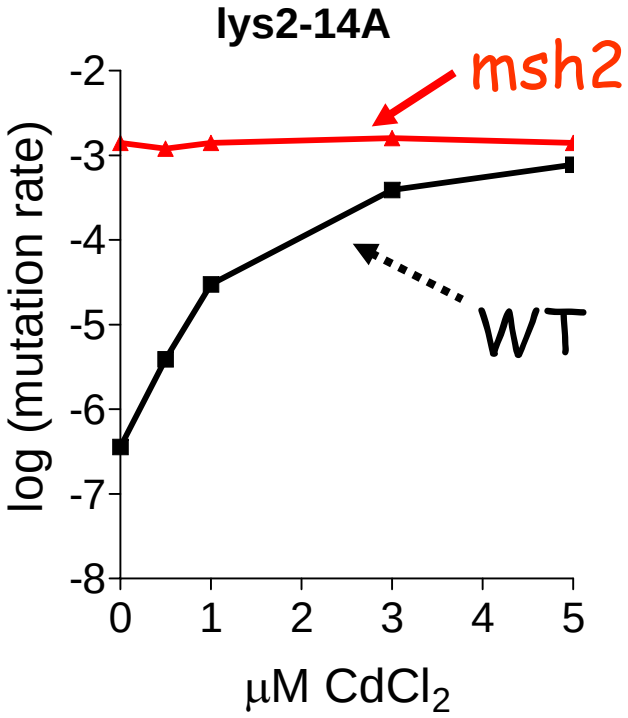


Cadmium inhibits DNA mismatch repair in extract from human cells



Cadmium is hyper-mutagenic (*as much as x2,000*) to wild type yeast cells

Cadmium is not mutagenic to yeast cells that are deficient in MMR (*msh2*)



Interaction between mutator effects of cadmium and MMR-null resembles epistatic interactions between two mutator defects in the same pathway.

How to prove that yeast MMR is *in vivo* target for cadmium ?

Compare wild type yeast grown on cadmium with MMR-deficient yeast mutants

High Risks of Genome Instability

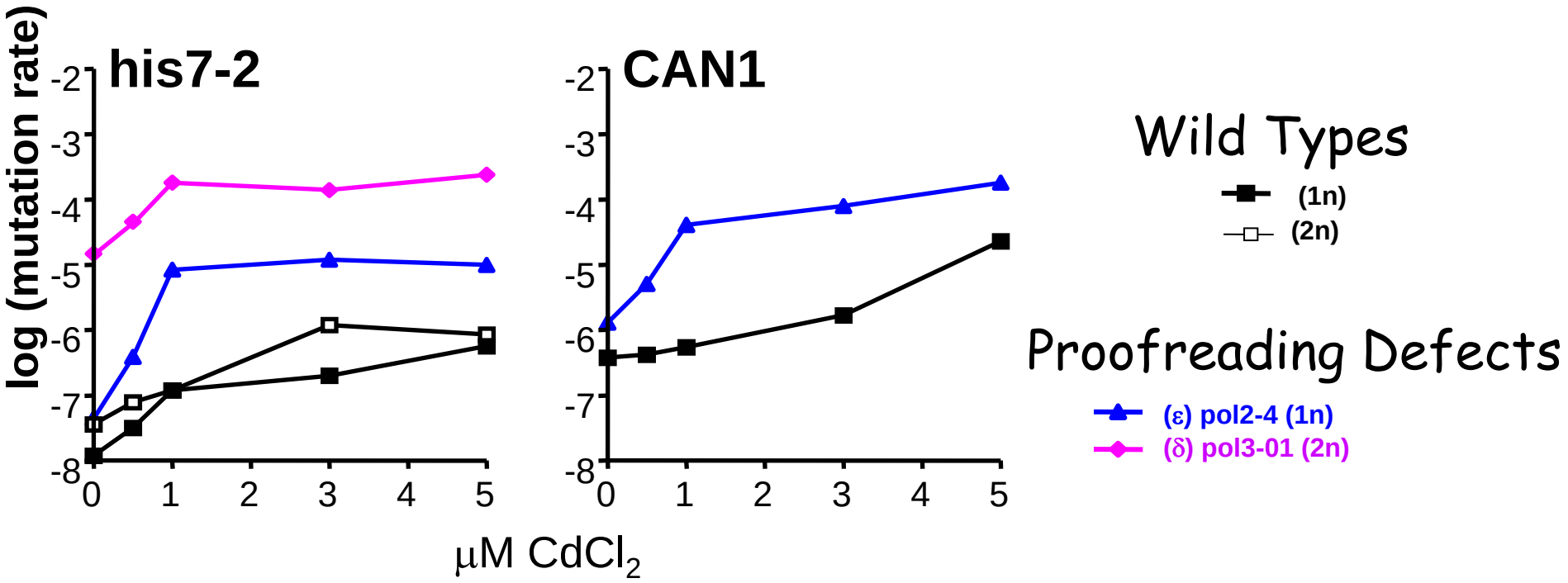
Combination of MMR and proofreading defects cause catastrophic mutability

(Morrison & Sugino; Schaaper)

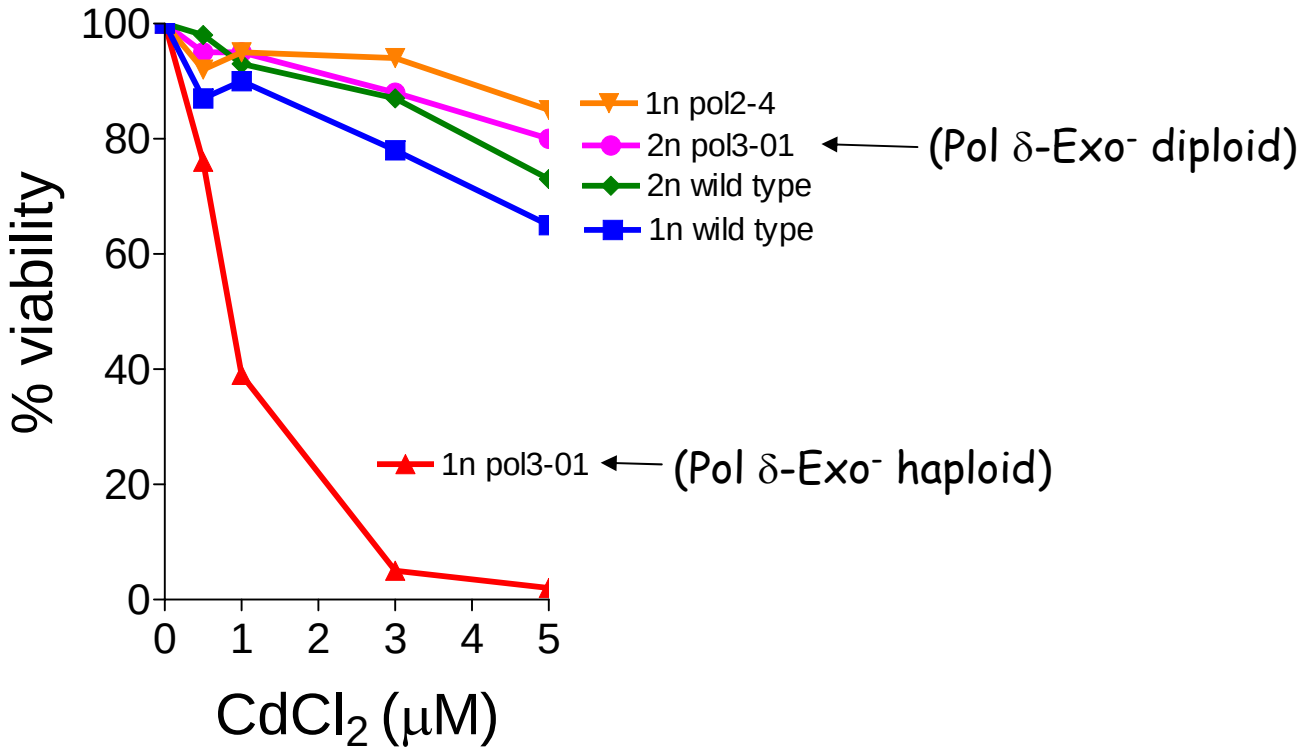
- lethality (error catastrophe) in haploids

- synergy between mutators

Cadmium Causes Synergistic Hyper-mutability Combined with Defects in Proofreading



Cadmium reduces viability of Pol δ Exo-deficient haploid (*Note: viability of isogenic diploids is not reduced*)



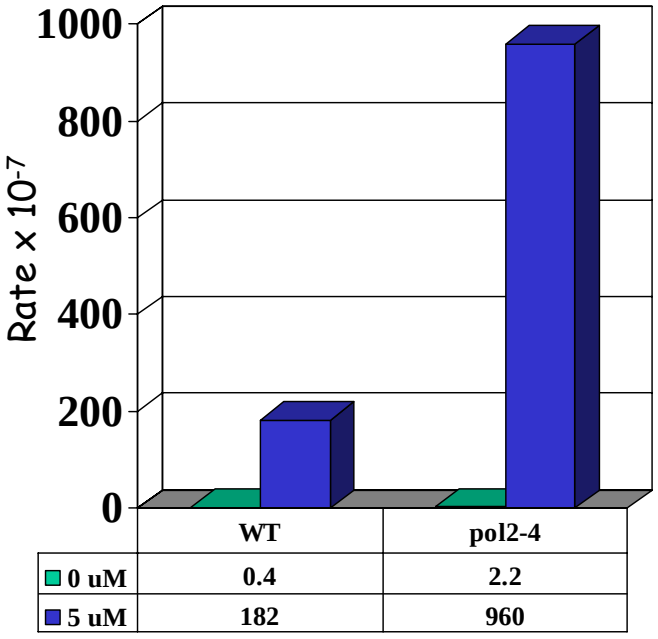
"Synthetic lethality" of cadmium with Pol δ Exo-deficiency can be due to catastrophic rate of recessive lethals.

Compare MMR-Deficient Yeast with Yeast Grown on Cadmium

MMR deficiency	Cadmium
Synergy with Proofreading Defects -hyper-mutability -synthetic lethality	yes

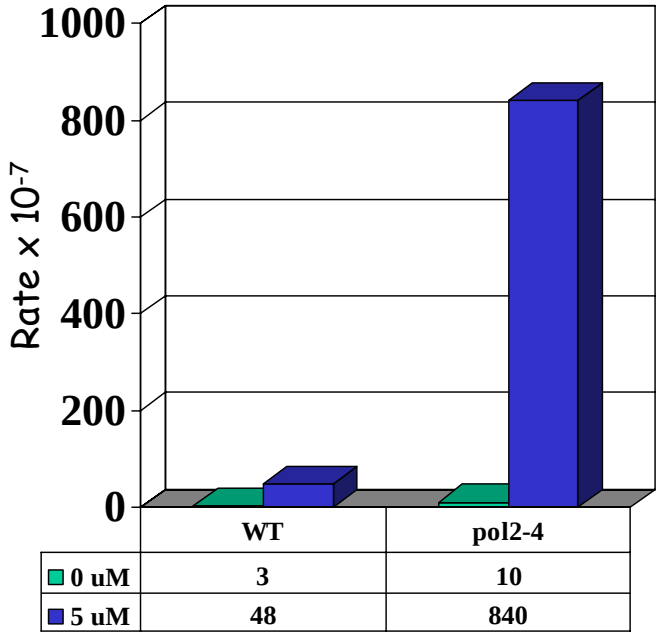
Cadmium Can Induce Base Substitutions and Frameshifts

Frameshifts



	WT	pol2-4
0 uM	0.4	2.2
5 uM	182	960

Base substitutions



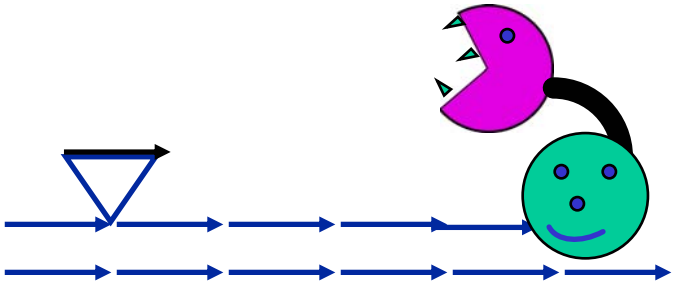
	WT	pol2-4
0 uM	3	10
5 uM	48	840

Mutations in the yeast *CAN1* gene

Compare MMR-Deficient Yeast with Yeast Grown on Cadmium

MMR deficiency	Cadmium
Synergy with Proofreading Defects -hyper-mutability -synthetic lethality	yes
Base substitutions and frameshifts	yes

At-Risk Motifs (ARMs) -- long homonucleotide runs and other microsatellites are poor substrates for the 3' →5' Exo of DNA Pol



Consequences:

- **homonucleotide runs are hyper-mutable in MMR-deficient cells**
- **mutation rate depends on the size of run and type of frameshift**

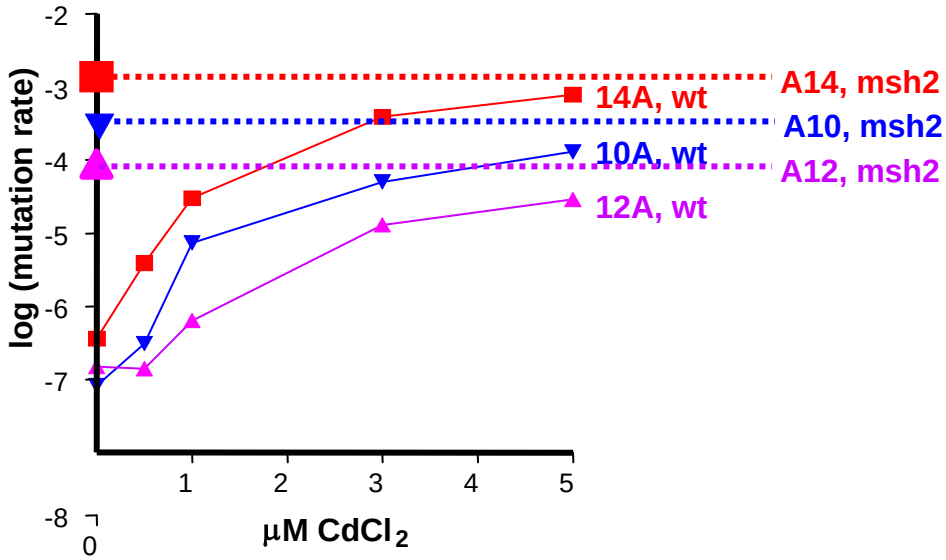
- homonucleotide runs are hyper-mutable in MMR-deficient cells

- mutation rate depends on the size of run and type of frameshift

Homonucleotide run (frameshift in run)	**Mutation rate ($x10^{-9}$)**		**mutator effect of the *msh2***
	Wild type	***msh2***	
A4 (-1 nt)	**0.4**	**31**	**x 78**
A7 (-1 nt)	**4**	**1,550**	**x 408**
A10 (-1 nt)	**47**	**314,000**	**x 6,681**
A14 (-1 nt)	**164**	**1,600,000**	**x 9,756**
A5 (+1 nt)	**1**	**37**	**x 34**
A8 (+1 nt)	**10**	**3,440**	**x 344**
A12 (+1 nt)	**190**	**84,000**	**x 444**

Size of a run and a type of frameshift:

Relative mutability of runs mimics MMR-deficiency

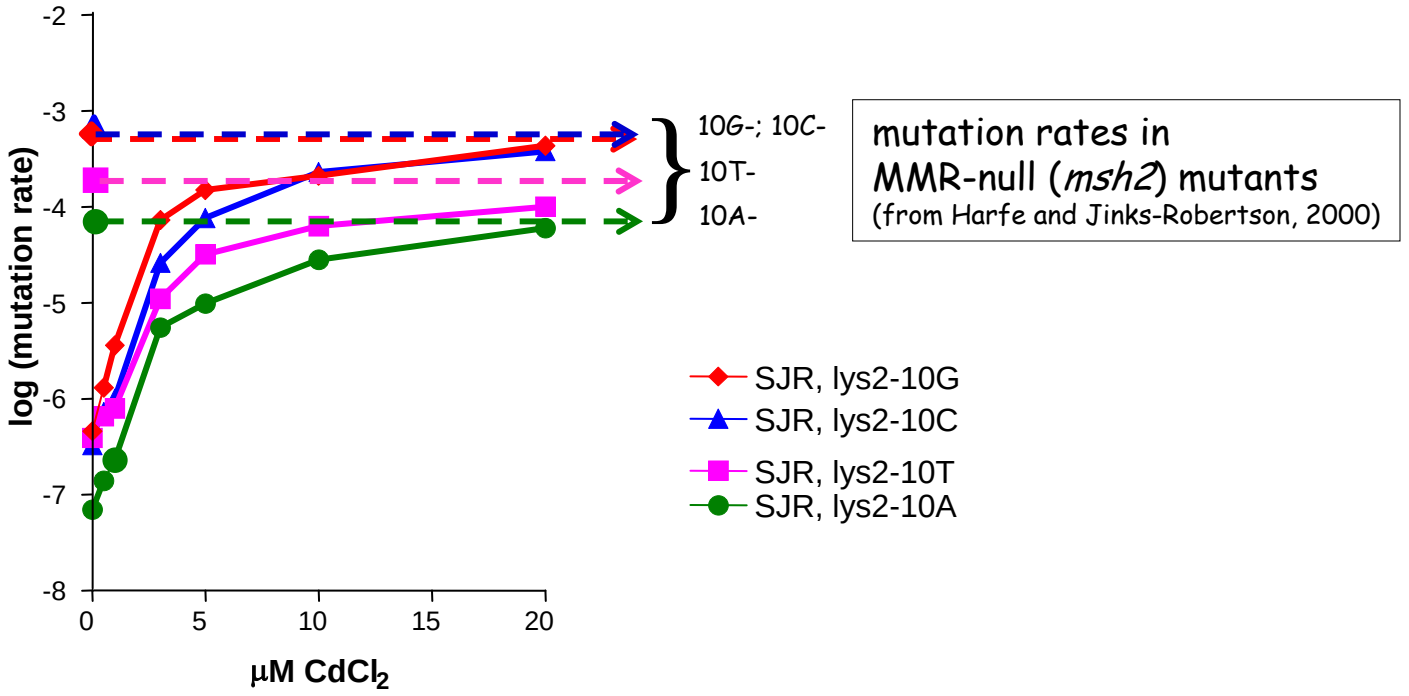


A14, -1 nt mutation

A10, -1 nt mutation

A12, +1 nt mutation

Relative mutability of G-, C-, T-, and A- runs mimics MMR-deficiency



Compare MMR-Deficient Yeast with Yeast Grown on Cadmium

MMR deficiency	Cadmium
Synergy with Proofreading Defects -hyper-mutability -synthetic lethality	yes
Base substitutions and frameshifts	yes
Mutator signature in homonuleotide runs: -type of frameshift -size of a run -sequence of a run	yes

Compare MMR-Deficient Yeast with Yeast Grown on Cadmium

MMR deficiency = Cadmium

Wild type yeast grown on cadmium behave as if they are deficient in mismatch repair

Hyper-mutability is caused by cadmium via inhibiting post-replication mismatch repair (MMR)

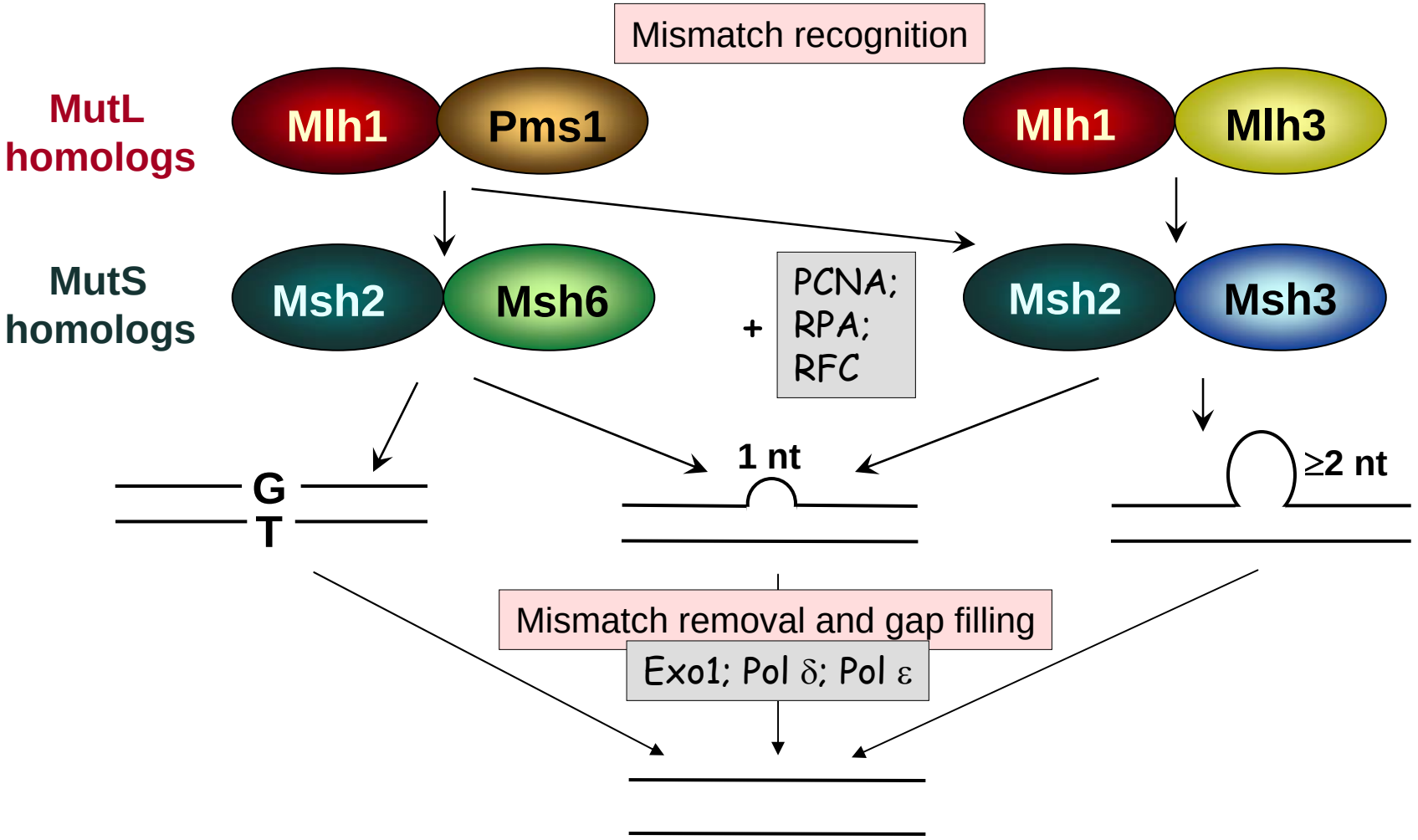
- Very low (micromolar) concentrations of Cd^{2+} ions, similar to those present in the environment and accumulated in organisms, inhibit MMR in yeast and human cell extract leaving about 20-50% mismatches unrepaired.

- Cadmium is a new kind of mutagen that causes hyper-mutability by inhibiting mutation avoidance DNA repair system, rather than by damaging DNA.

Specific notes of relevance:

- Cadmium is common in the environment.
- MMR system prevents mutations and cancer.

What is the target for cadmium inside eukaryotic MMR?



OTHER QUESTIONS:

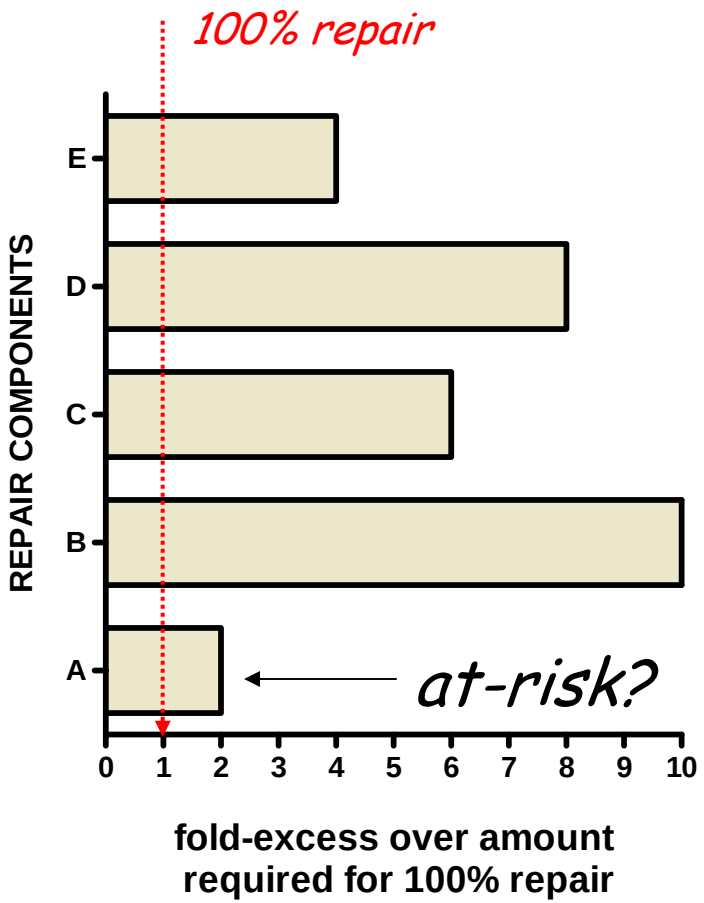
- Effect of cadmium on MMR in other species
  (*pathogenic fungi*, *adaptive changes, evolution*)
- Effect of cadmium on other functions of MMR proteins
  (*meiosis, recombination, apoptosis, damage recognition, tolerance to alkylation damage*)
- Effect of cadmium on mammalian MMR
  (*species, cell types, tissues, genotypes, relation to carcinogenesis*).
- Other environmental factors and drugs that could inhibit MMR
- Mutagenesis caused by inhibiting other repair and fidelity systems with environmental factors and drugs

Mutagenesis caused by inhibiting repair and fidelity systems by environmental factors and drugs

Where to look for targets?

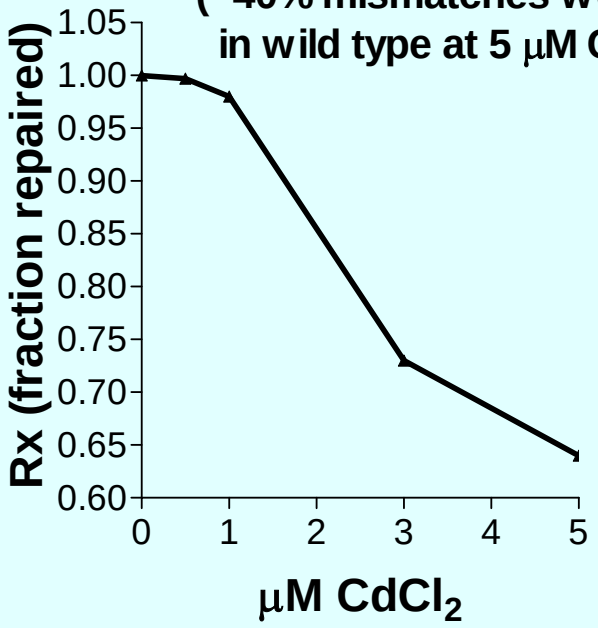
- *Identify sensitized motifs in protein structures*
- *Identify repair components present at the "threshold" level*

Identify repair components present at the "threshold" level



Repaired fraction of mismatches at different concentrations of cadmium.

(~40% mismatches were not repaired in wild type at 5 μM $CdCl_2$



Cadmium acts as a mutagen by inhibiting mismatch repair.
(Genotoxicity caused by inhibition of a mutation avoidance system)

Dmitry A. Gordenin
National Institute of Environmental Health Sciences (NIEHS)

COLLABORATORS (all from NIEHS):

Yong Hwan (Yohan) Jin
Hanan Al-Refai
Michael Resnick

Alan Clark
Tom Kunkel

Rob Slebos
Jack Taylor