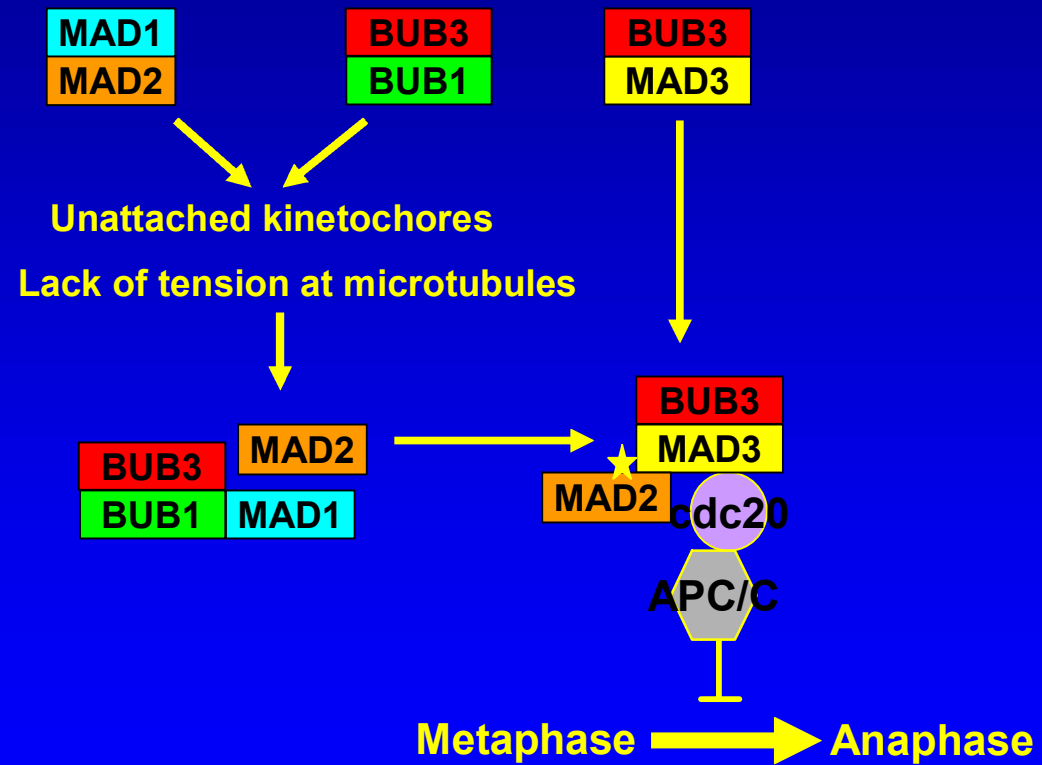
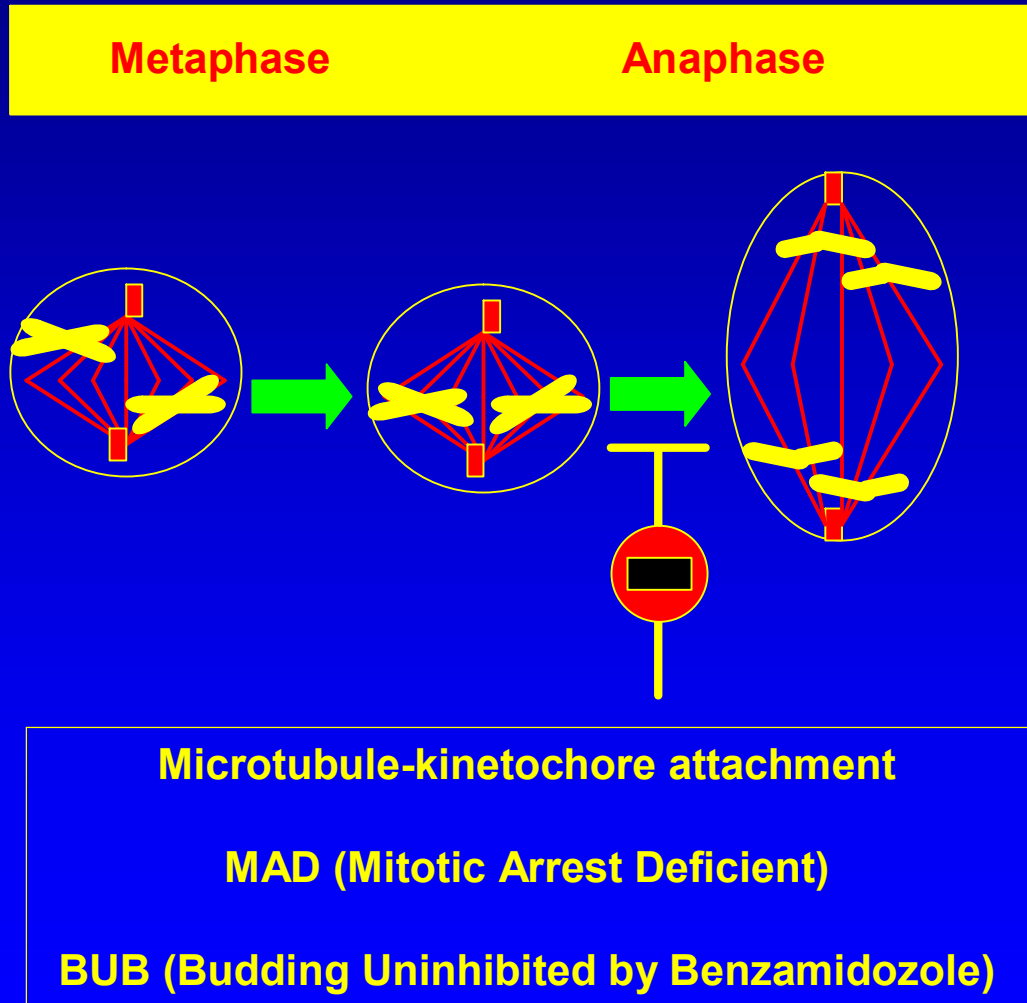


**Functional analysis of colorectal
tumour associated deletions in
S. cerevisiae spindle checkpoint
genes *bub1* and *mad3***

Susan M Farrington

University of Edinburgh

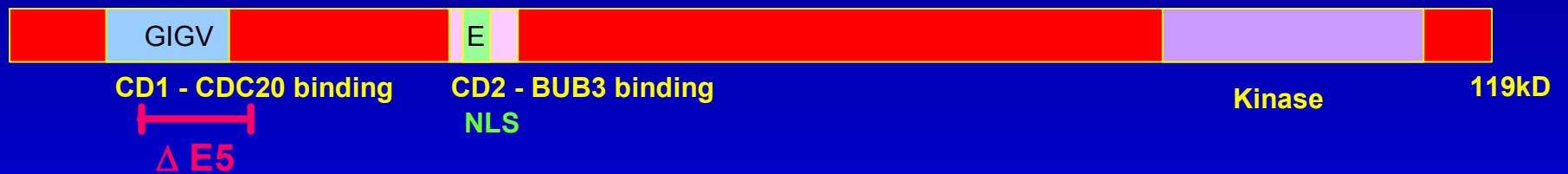
Mitotic Checkpoints prevent mis-segregation



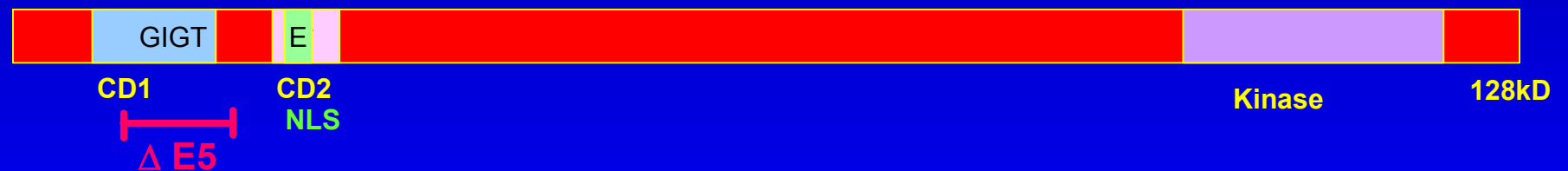
Tumour mutations of BUBR1

- Aneuploid tumour RNA screened for BUB1 and BUBR1
- Deletion of BUBR1 identified in 30% of tumours

BUBR1



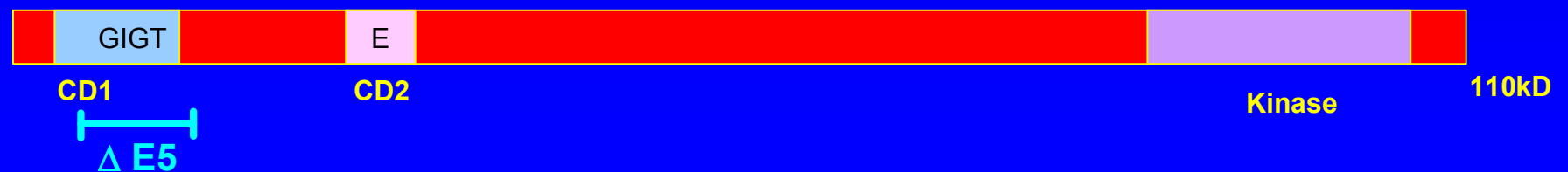
BUB1



YEAST MAD3 – orthologue of BUBR1

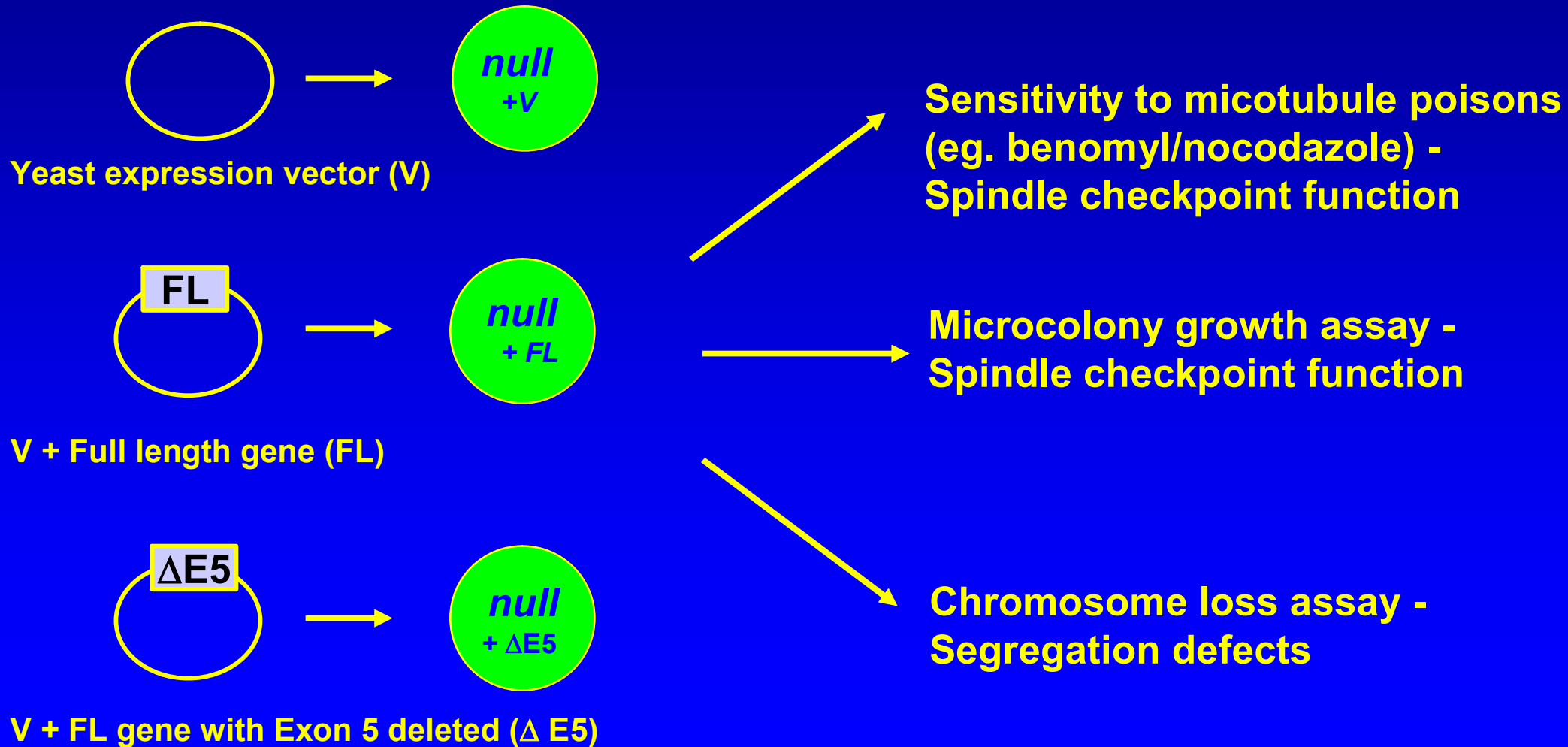


YEAST BUB1



Functional analysis in *mad3/bub1* null strains

Transformation of *mad3* or *bub1* null strains



Spindle Checkpoint function

MAD3

100 10 1



vector (V)
deletion ($\Delta E5$)
full length (FL)
mad3⁻
WT

YPD +
12.5 μ g/ml
benomyl

$\Delta E5$ is benomyl sensitive
Does not rescue null phenotype
No checkpoint function

BUB1

100 10 1

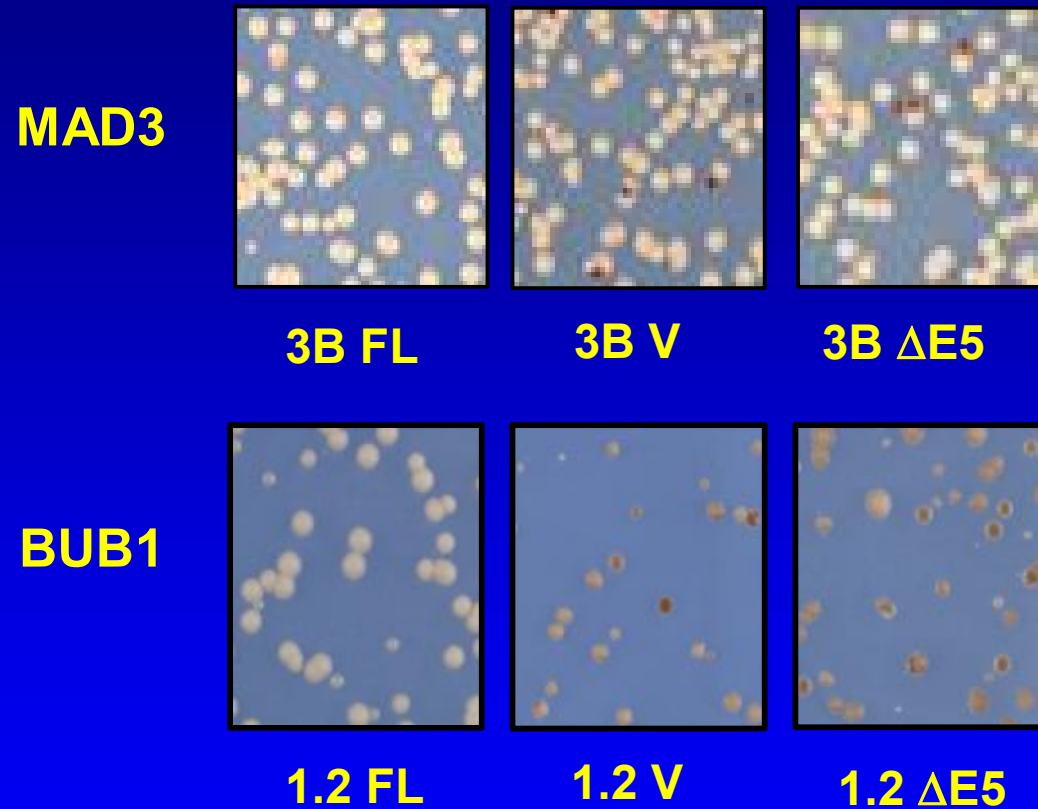


vector (V)
deletion ($\Delta E5$)
full length (FL)
bub1⁻
WT

YPD +
12.5 μ g/ml
benomyl

$\Delta E5$ is benomyl sensitive
Partially rescues null phenotype
Retains partial function.

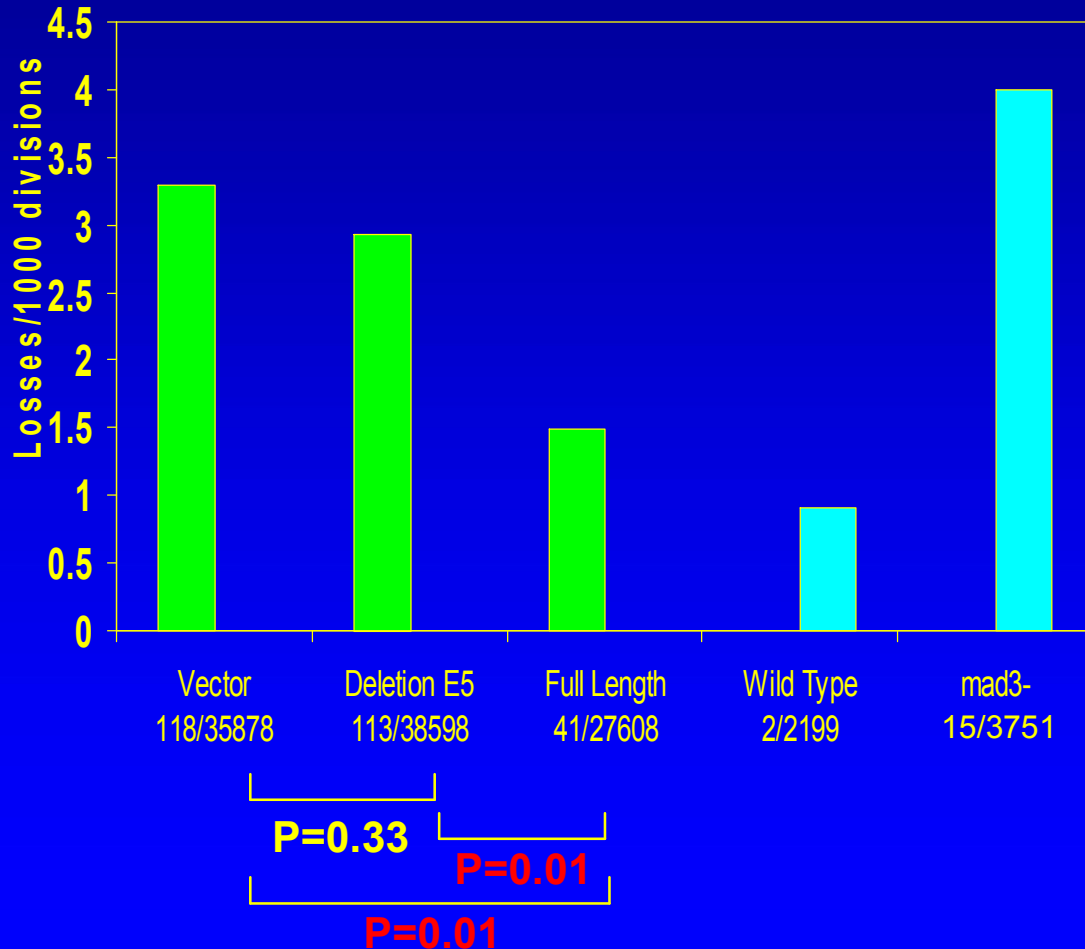
Chromosome segregation analysis



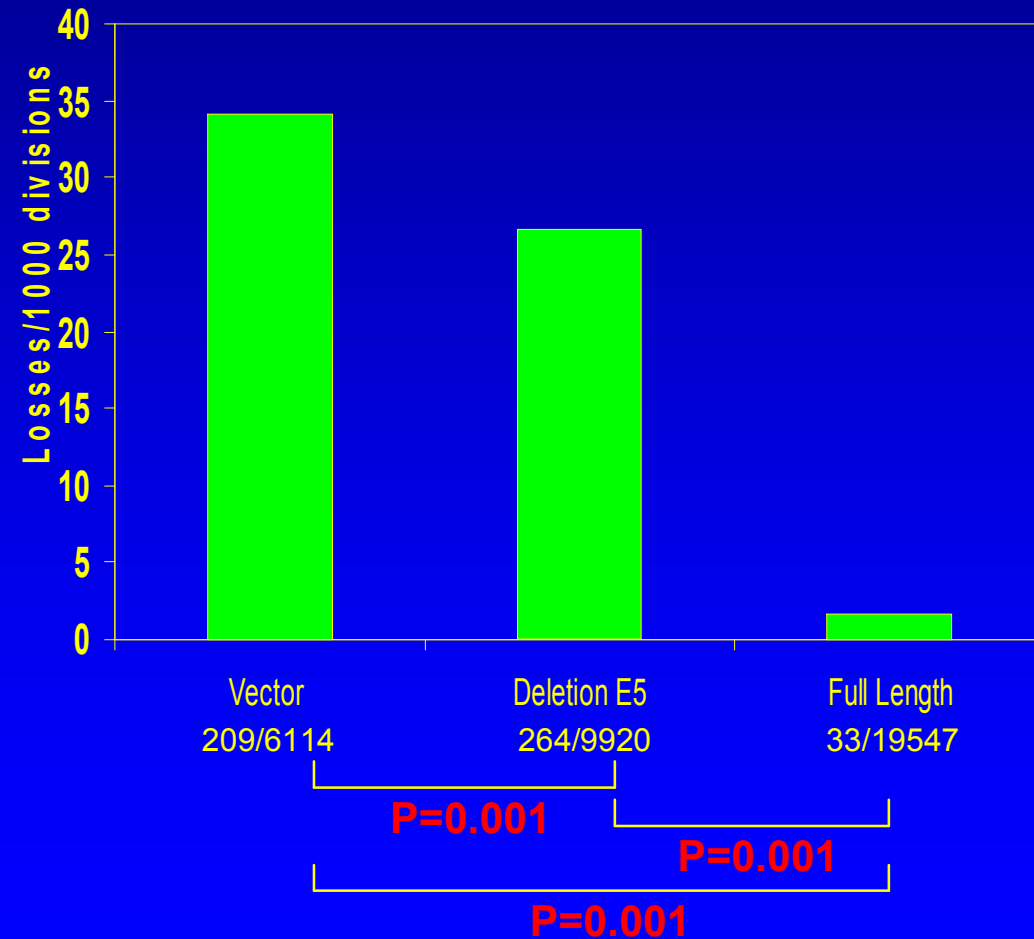
Chromosome Loss Assay - measures loss of a mini-chromosome at first mitotic division

$\Delta E5$ displays segregation defect

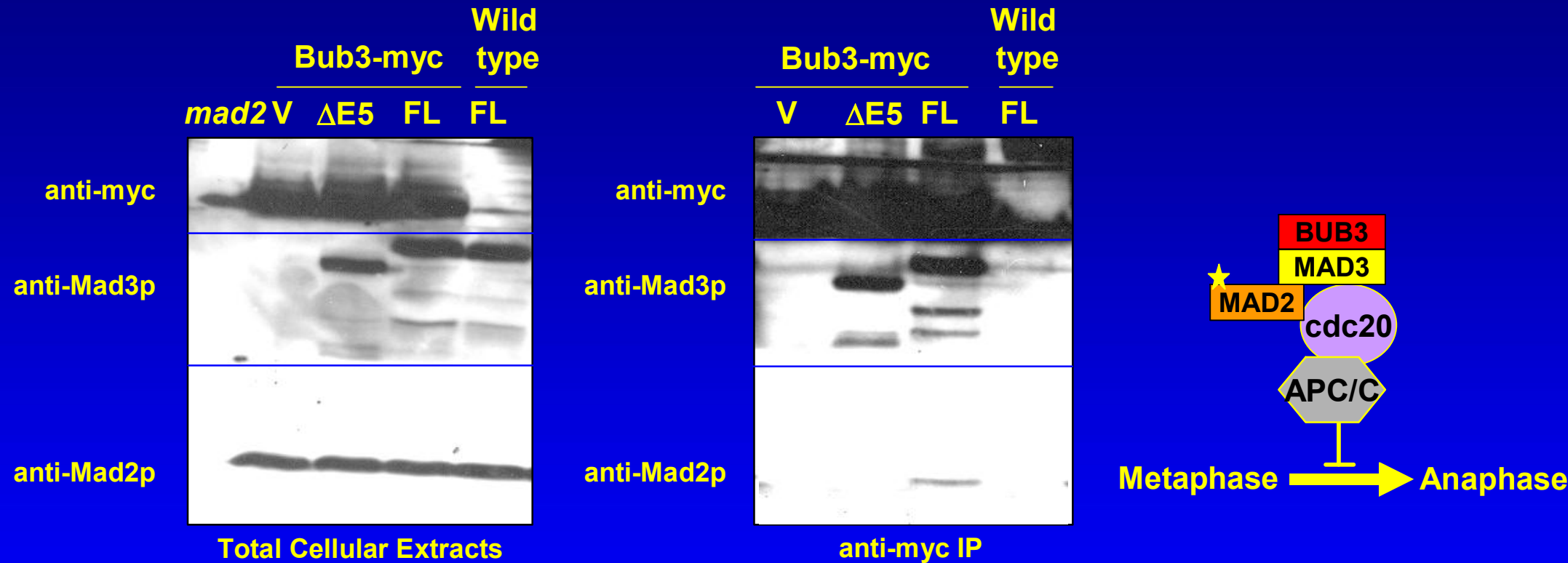
MAD3



BUB1



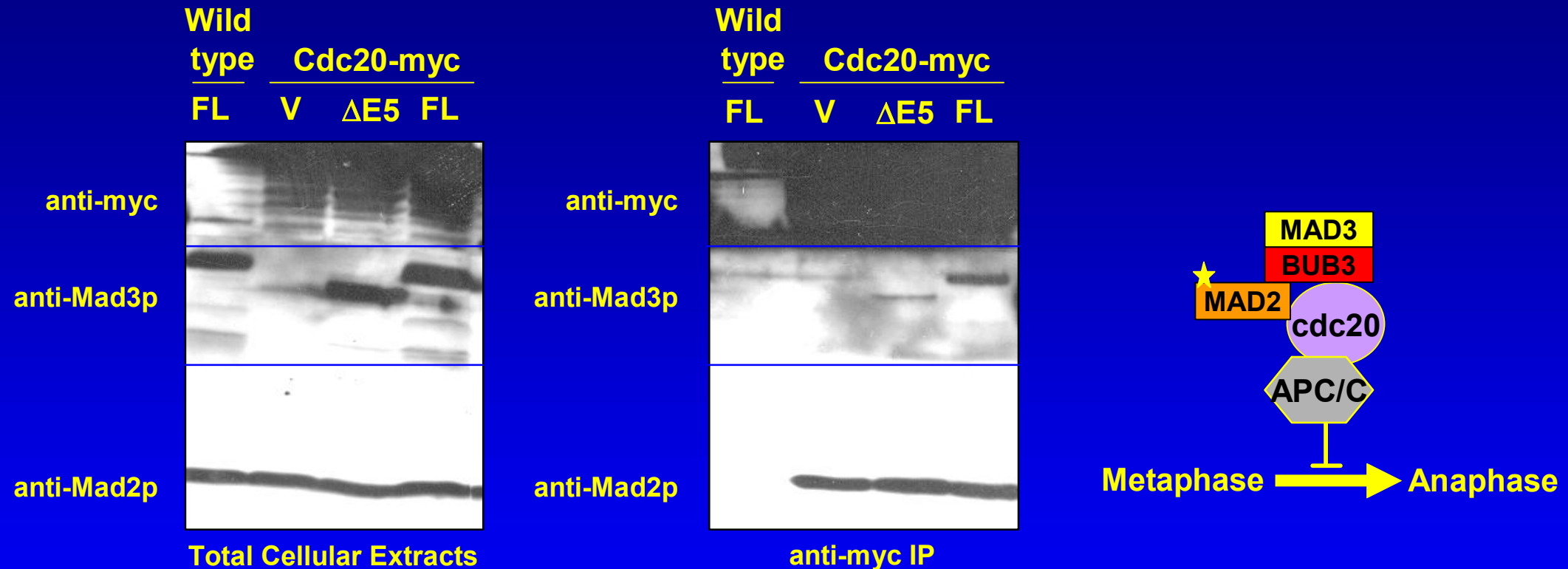
Mad3 Δ E5p reduces Bub3p/Mad2p interactions



Δ E5 causes a reduction in - Bub3p binding
- Mad2p binding

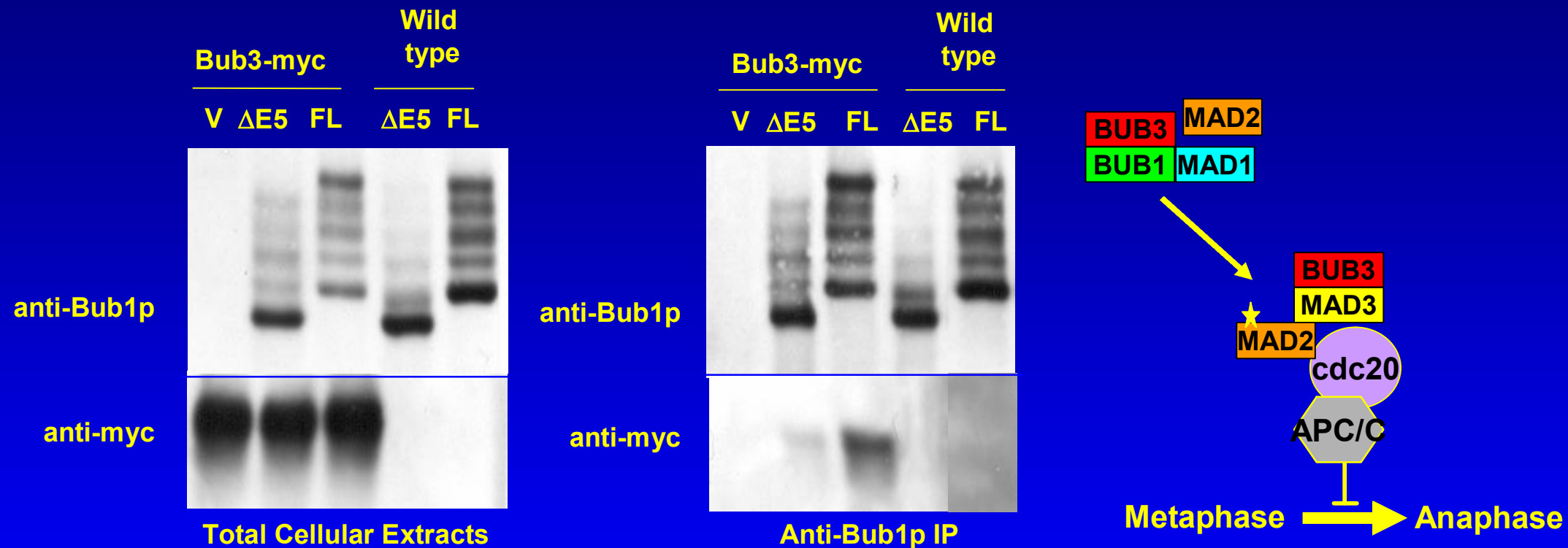
Suggests Mad3p required for complex of Bub3p and Mad2p

Mad3 Δ E5p reduces Cdc20p interactions



Δ E5 causes a reduction in Cdc20p binding
Mad3p does not interact directly with Mad2p
and not required for Mad2p/Cdc20p interaction

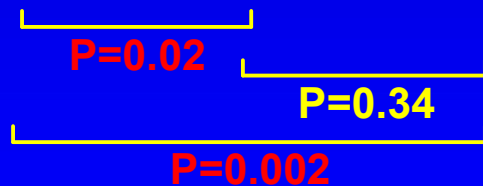
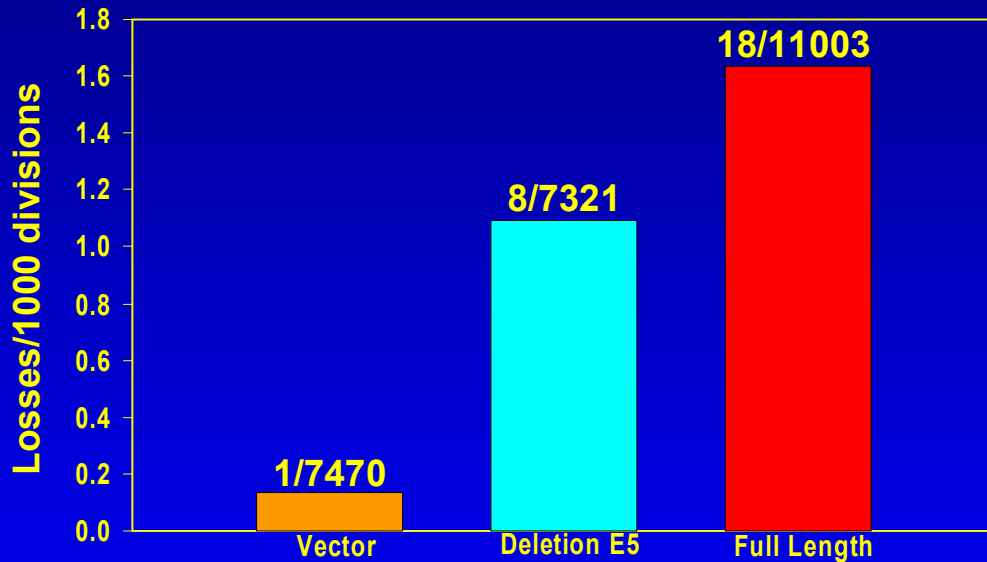
Bub1 Δ E5p reduces Bub3p/Cdc20p interactions



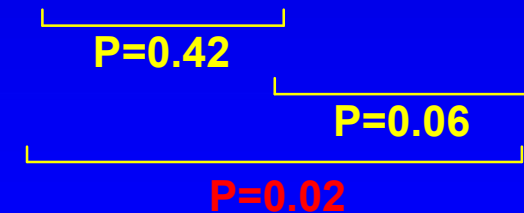
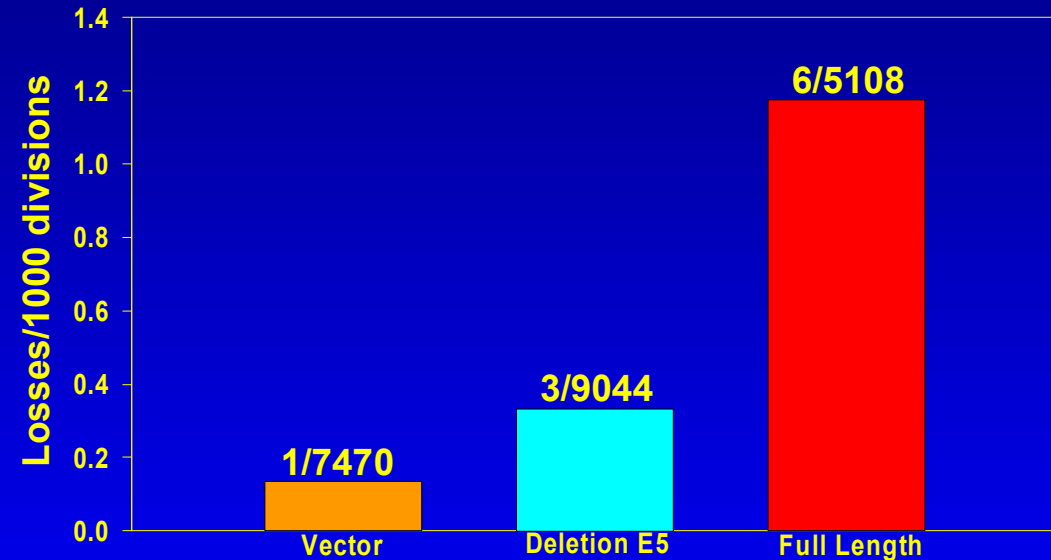
- Δ E5 causes - reduction in Bub3p binding by about 60%
- reduction in Cdc20p binding (data not shown)
- decrease in the phosphorylation of Bub1p

Dominant-negative effect of $\Delta E5$

mad3 expression in wild-type strain



bub1 expression in wild-type strain



**Mad3 $\Delta E5$ displays a dominant-negative effect
whereas this is not obvious in Bub1 $\Delta E5$**

Summary

- Mad3p required for Mad2p/Bub3p complex, although not direct interaction and not required for Mad2p/Cdc20p
- $\Delta E5$ abrogates checkpoint function and shows segregation defect
- $\Delta E5$ in both Mad3p and Bub1p causes some reduction of Bub3p and Cdc20p binding
- $\Delta E5$ in Bub1p reduces phosphorylation
- $\Delta E5$ in Mad3p displays a dominant negative effect

No genomic mutation identified so far suggesting possible alternate splice form



Acknowledgements



PEOPLE

Laura Boyes

Malcolm Dunlop

Kevin Hardwick

Hardwick Lab

Colon Cancer Genetics Group

FUNDING BODIES

Chief Scientist Office of Scotland

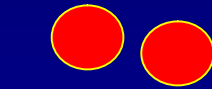
Medical Research Council

Royal Society of Edinburgh

Cancer Research UK



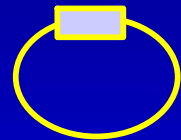
Colour Colony Mini-chromosome Loss Assay



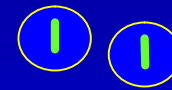
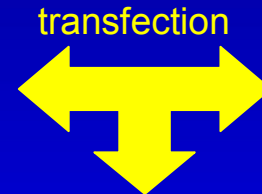
ADE2-101
(ochre mutation in
ADE2 gene)



Minichromosome with
SUP11 tRNA gene (ochre
suppressing)



Mad 3 deletion
construct



ADE2-101
+ SUP11 tRNA

select white colonies

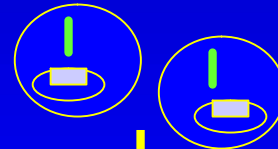
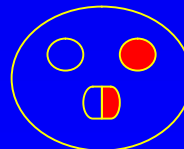


Plate (- adenine)

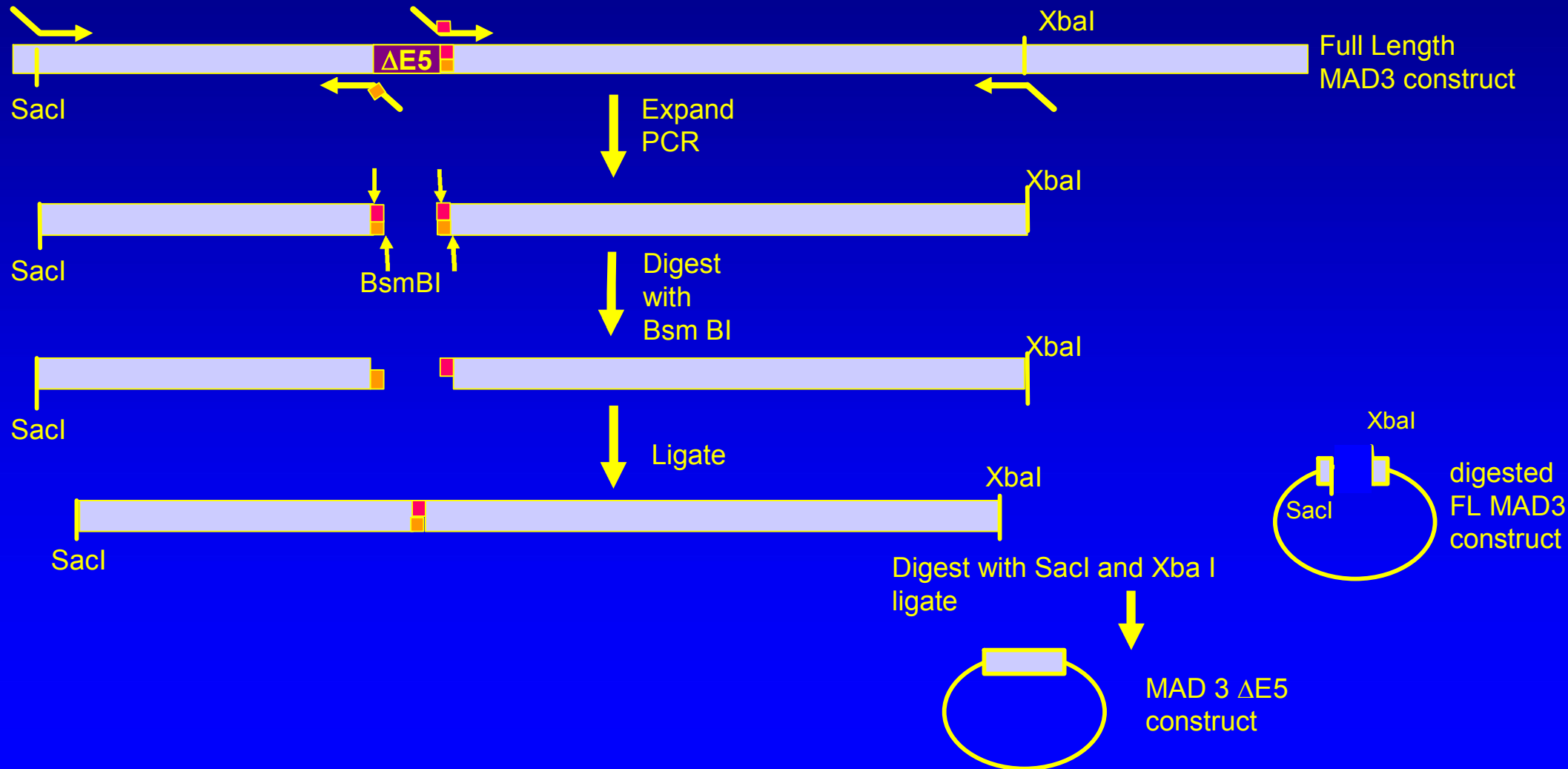


Represents Minichromosome loss at
first division after plating



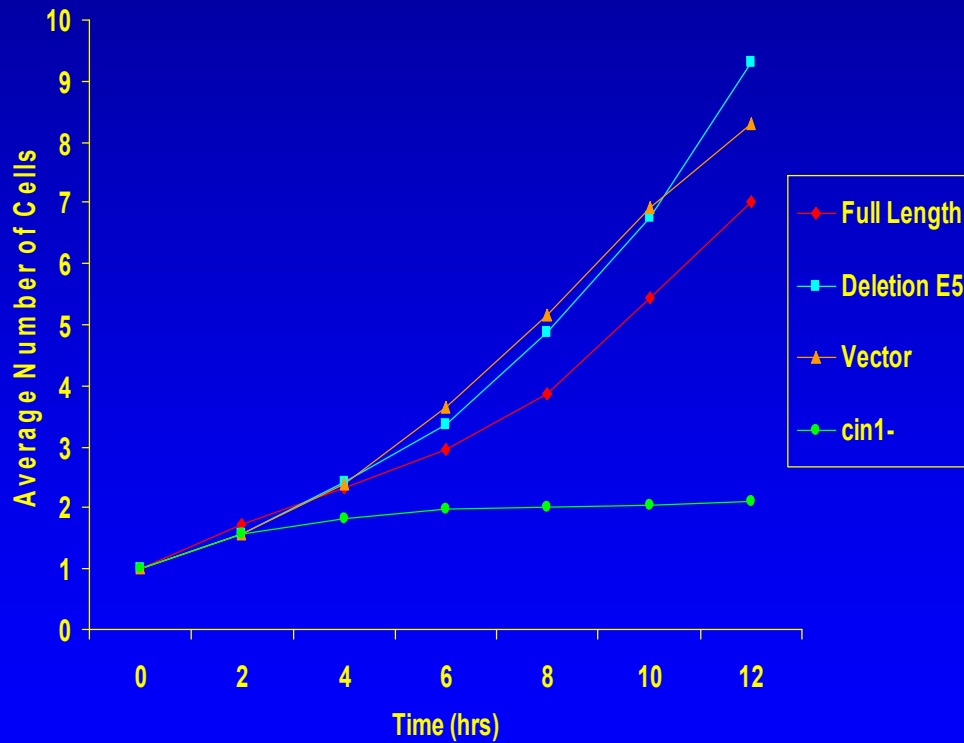
Chromosome loss frequency = half-sectored colonies / mostly white colonies

Synthesis of $\Delta E5$ Constructs

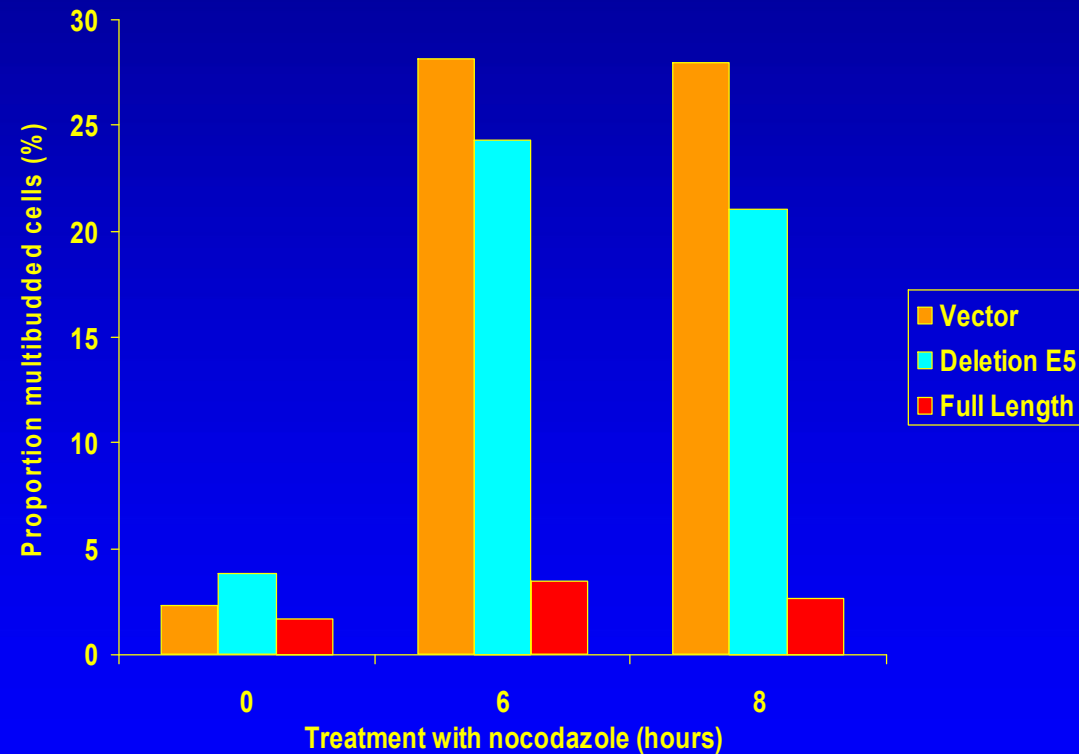


Checkpoint or spindle defects

MAD3 – Microcolony assay



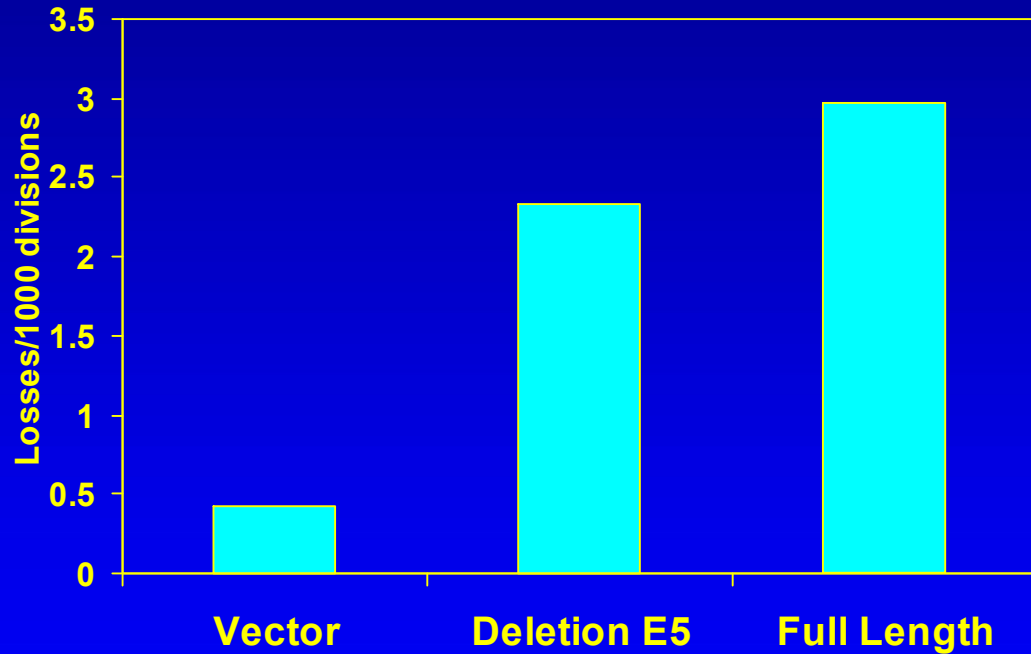
BUB1 – Liquid culture assay



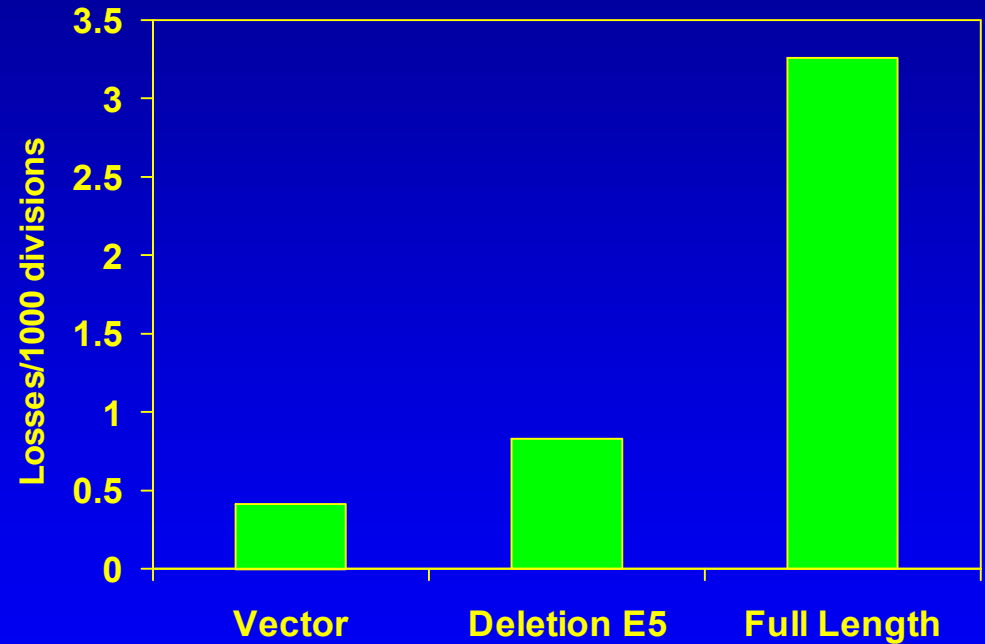
Checkpoint mutants display no delay in growth – similar to null phenotype

Bub3 binding analysis via overexpression

MAD3



BUB1



Overexpression studies demonstrate that $Mad3\Delta E5$ retains some Bub3p binding, whereas $Bub1\Delta E5$ has greatly reduced Bub3p binding

α -myc IP

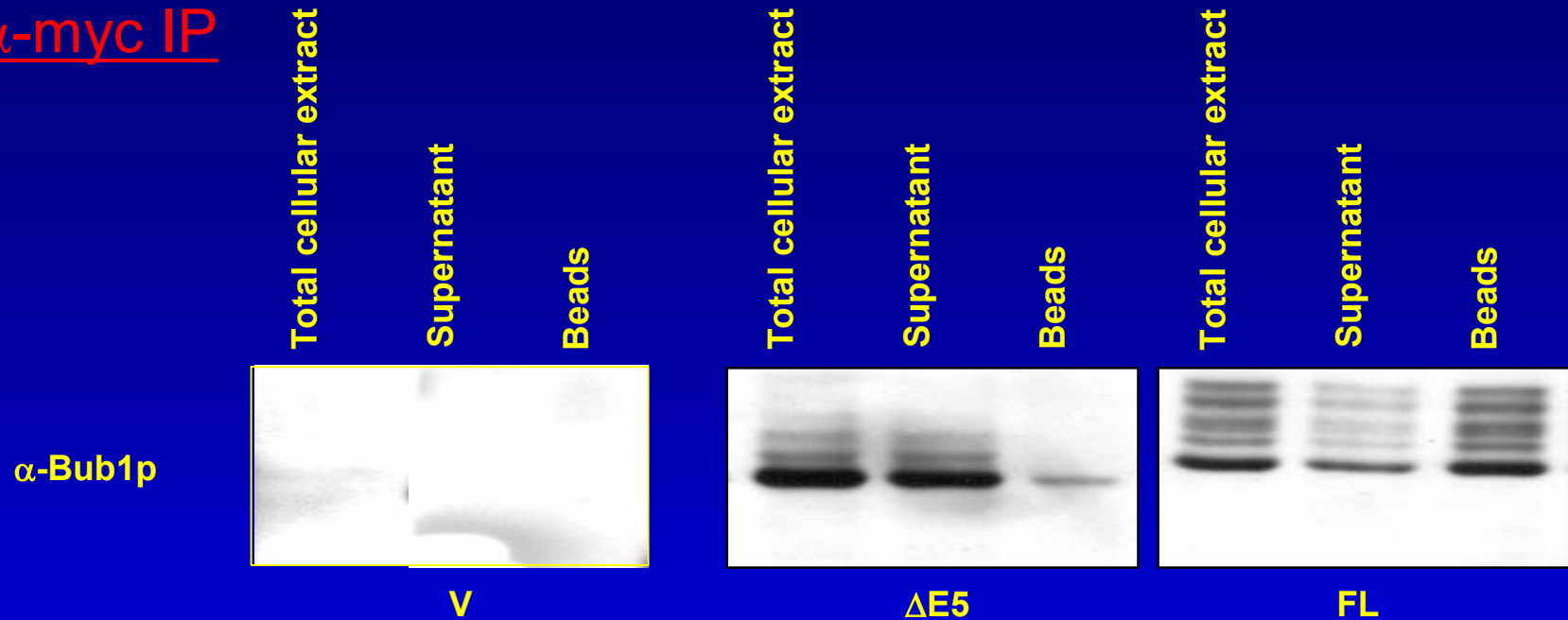


Figure 5.5 Initial data suggest Bub1 $\Delta E5$ p may form reduced interactions with Cdc20p

A roughly equivalent amount of protein is observed in the total cellular extracts isolated from both the $\Delta E5$ and full-length transformants. However, the amount of Bub1p which immunoprecipitates with myc-tagged Cdc20p is reduced in the $\Delta E5$ mutant indicative of a reduction in the Cdc20p binding activity of Bub1 $\Delta E5$ p compared to the full-length protein. Consistent with this, the amount of Bub1p in the $\Delta E5$ supernatant fraction is raised compared to the full-length fraction.