Office hours
Wednesday 3-4pm
304A Stanley Hall

Review session
5pm Thursday, Dec. 11
GPB100

Coding sequence array

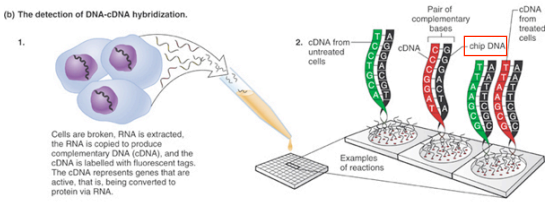
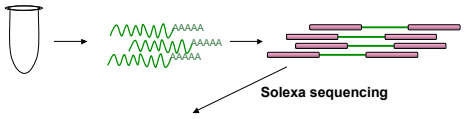


Fig. 1.13

"RNA-seq"



counts	seq	maps	genome	pos	orient
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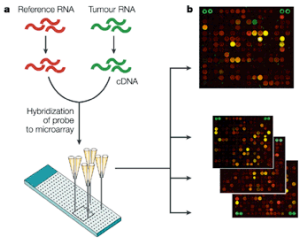
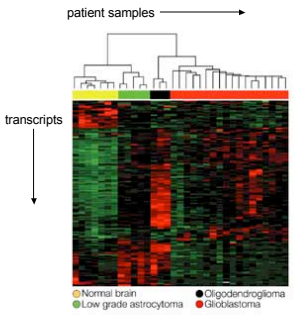
25885	TCTCTACTGGTGGTGGT	U0	chr03.fsa	138912	F
17851	GACAAACTGTCGCTGTC	R0			
15715	TCTCTACCGGTGGTGGT	U0	sbaySum.fsa	11182634	F
14963	TCGTTCACTTGTAATGG	U0	chr04.fsa	1489376	R
...					
610	TTGAAGCTACCACCAAC	R0			
607	TGTCCTTATCTATGTGT	U0	sbaySum.fsa	8491512	R
602	CGTACTTTTTTAATTGT	U1	chr11.fsa	145622	F
602	ATCATTGTTCCAGAAAT	R0			
601	TTATCAGAGGTGCTGGT	U0	chr14.fsa	694435	R
600	CAAAGACCTCATTTAAT	U0	sbaySum.fsa	4671591	R
599	AGGAGGGTATATATGCT	U0	chr14.fsa	575509	R
...					

Expression effects of cancer

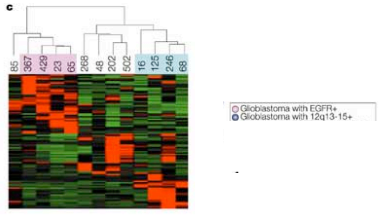


Microarray and histopathological analysis of tumours: the future and the past?

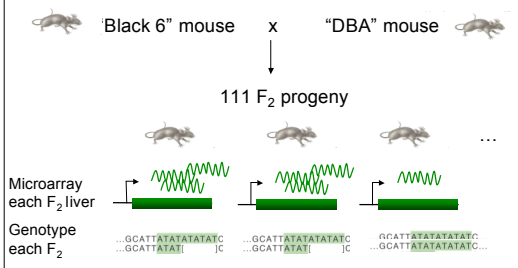
Diagnosis via transcriptional profile



Diagnosis via transcriptional profile

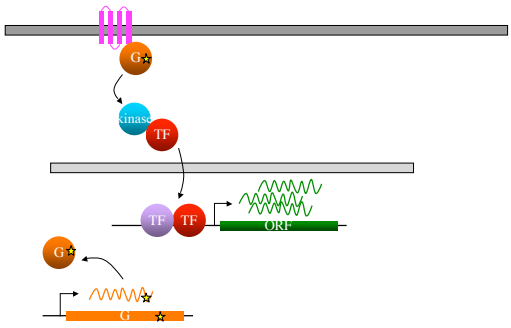


Linkage mapping of mRNA levels

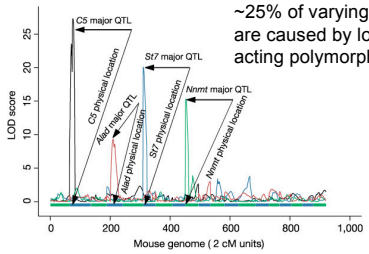


Looking for linkage (coinheritance) between marker and mRNA level.

Marker is linked to polymorphism in expression regulation cascade



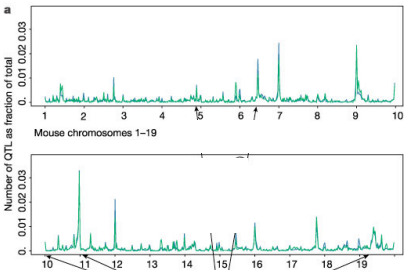
Locally acting polymorphisms



~25% of varying mRNAs are caused by locally acting polymorphism

Polymorphism responsible for mRNA difference is at the locus of the gene itself

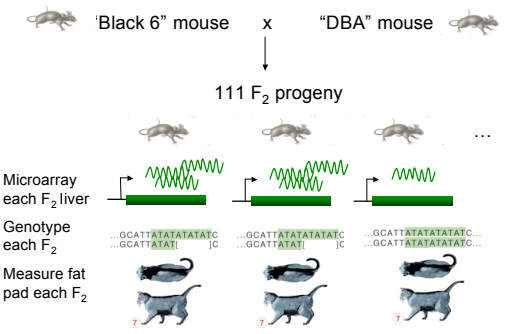
Nonlocal polymorphisms



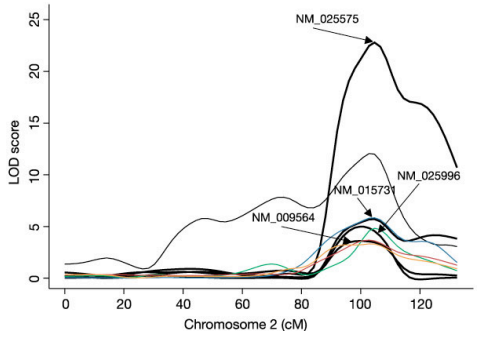
Nonlocal polymorphisms

One polymorphism in a key regulator can affect a regulon: 100's of related mRNAs.

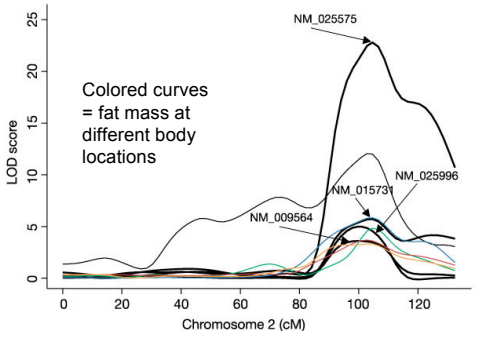
Clinical applications



Clinical applications



Clinical applications



Clinical applications

Finding polymorphism responsible for difference in macroscopic phenotype is hard

Clinical applications

Finding polymorphism responsible for difference in macroscopic phenotype is hard

If mRNAs change too, can learn mechanism from known function of encoded proteins

Clinical applications

Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice

Iiris Hovatta[1], Richard S. Tennant[1], Julie A. Ellison[1], Eric E. Schadt[1], Ito...

Identification of *Abcc6* as the major causal gene for dystrophic cardiac calcification in mice through integrative genomics

...s-Drake[3], Eric E. Schadt[1],

Integrating genotypic and expression data in a segregating mouse population to identify 5-lipoxygenase as a susceptibility gene for obesity and bone traits

Margarete Mehrabian[1], Hooman Allayee[2], Jirina Stockton[1], Pek Yee Lum[3], Thomas A Drake[4], Lawrence W Castellani[1], Michael Suh[1], Christopher Armour[3], Stephen Edwards[3], John Lamb[3], Aldons J Lusis[1,5–7] & Eric E Schadt[3]

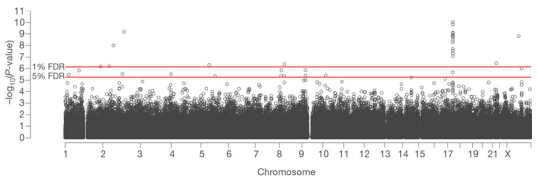
Clinical applications

Genetic variants regulating *ORMDL3* expression contribute to the risk of childhood asthma

Miriam F. Moffatt[1]*, Michael Kabesch[2]*, Liming Liang[3,4], Anna L. Dixon[1], David Strachan[5], Simon Heath[6], Martin Depner[7], Andrea von Berg[8], Albrecht Bufe[9], Ernst Rietschel[7], Andrea Heinzmann[10], Burkard Simma[11], Thomas Frischer[12], Saffron A. G. Willis-Owen[1], Kenny C. C. Wong[1], Thomas Illig[13], Christian Vogelberg[14], Stephan K. Weiland[15], Erika von Mutius[16], Gonçalo R. Abecasis[3], Martin Farrall[6], Ivo G. Gut[17], G. Mark Lathrop[17] & William O. C. Cookson[1]

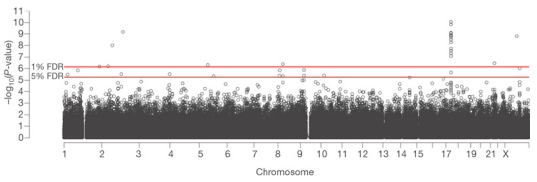
Asthma is caused by a combination of poorly understood genetic and environmental factors[1,2]. We have systematically mapped the effects of single nucleotide polymorphisms (SNPs) on the presence of childhood onset asthma by genome-wide association. We characterized more than 317,000 SNPs in DNA from 994 patients with childhood onset asthma and 1,243 non-asthmatics, using family and case-referent panels. Here we show multiple markers on chromosome 17q21 to be strongly and reproducibly associated with childhood onset asthma in family and case-referent panels with a combined P-value of $P < 10^{-6}$. In independent replication studies the 17q21 locus showed strong association with diagnosis of childhood asthma in 2,320 subjects from a cohort of German children ($P = 0.0003$) and in 3,301 subjects from the British 1958 Birth Cohort ($P = 0.0005$). We systematically evaluated the relationships between markers of the 17q21 locus and transcript levels of genes in Epstein–Barr virus (EBV)-transformed lymphoblastoid cell lines from children in the asthma family panel used in our association study. The SNPs associated with childhood asthma were consistently and strongly associated ($P < 10^{-15}$) in *cis* with transcript levels of *ORMDL3*, a member of a gene family that encodes transmembrane proteins anchored in the endoplasmic reticulum[3]. The results indicate that genetic variants regulating *ORMDL3* expression are determinants of susceptibility to childhood asthma.

Clinical applications

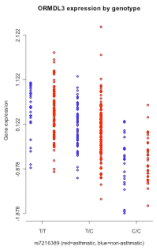


Clinical applications

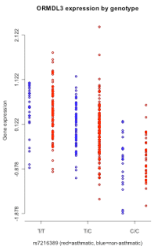
$$\frac{\text{Counts allele 1/allele 2, cases}}{\text{Counts allele 1/allele 2, controls}} = 1.5$$



Clinical applications



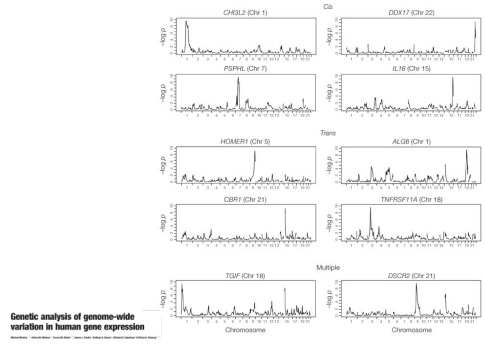
Clinical applications



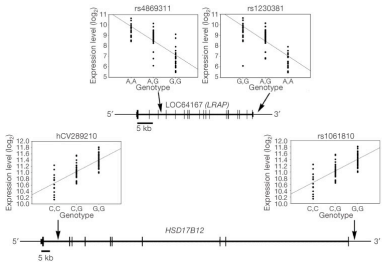
Marker predicts quantitative expression level = association

Can we map expression traits first, disease afterward?

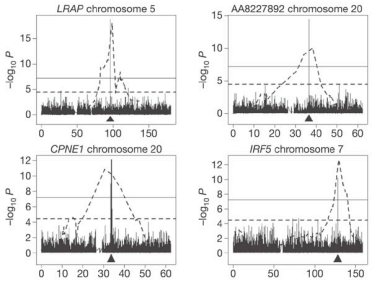
Linkage of human transcripts



Linkage of human transcripts



Association of human transcripts



Mapping determinants of human gene expression by regional and genome-wide association

Vivian G. Cheung[1,2], Richard S. Spielman[1], Kathryn G. Ewens[1], Teresa M. Weber[1,2], Michael Morley[1] & Joshua T. Burdick[1]

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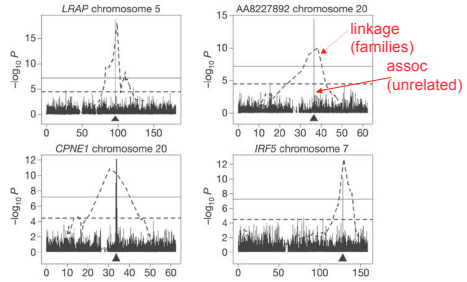

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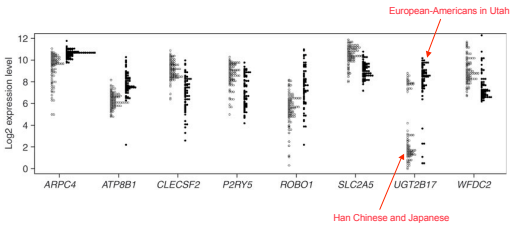
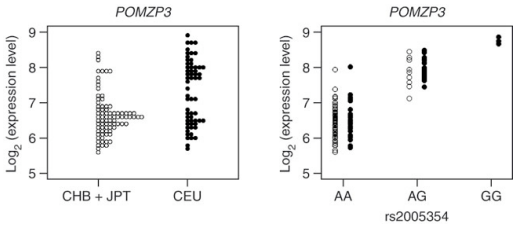
Association in multiple populations



Common genetic variants account for differences in gene expression among ethnic groups

Richard S Spielman[1], Laurel A Bastone[1], Joshua T Burdick[1], Michael Morley[1], Warren J Ewens[1] & Vivian G Cheung[1,2]

Association in multiple populations



A new brand of genetic variation

Translation

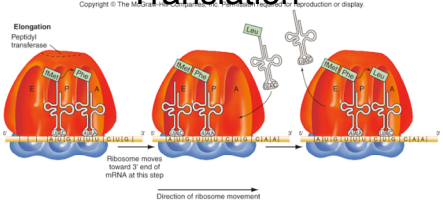


Fig. 8.25

Translation

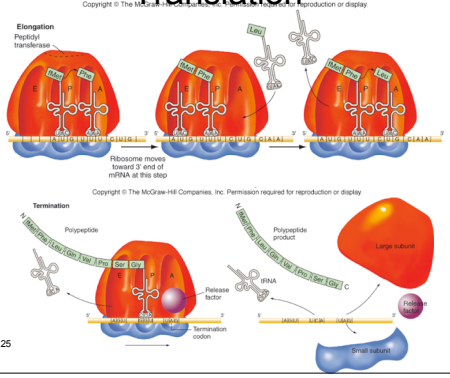
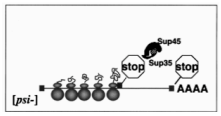


Fig. 8.25

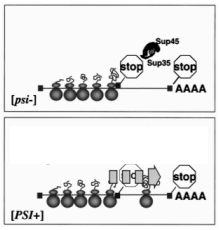
PSI+ yeast read through STOPs



[*PSI*⁺], *SUP35*, AND CHAPERONES

TRICIA R. SERIO* AND SUSAN L. LINDQUIST†

PSI+ yeast read through STOPs



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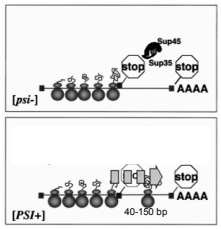

[*PSI*⁺], *SUP35*, AND CHAPERONES

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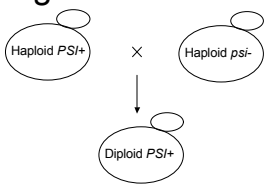
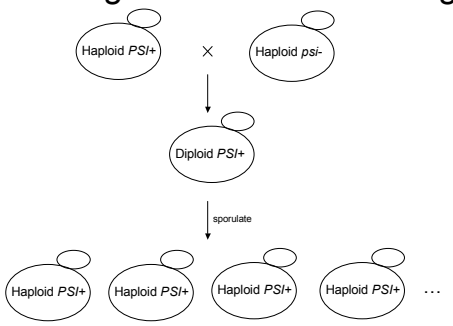
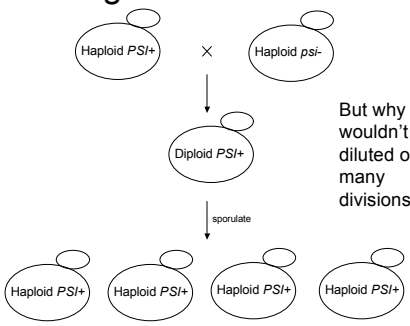
Weird genetics of read-through



Weird genetics of read-through

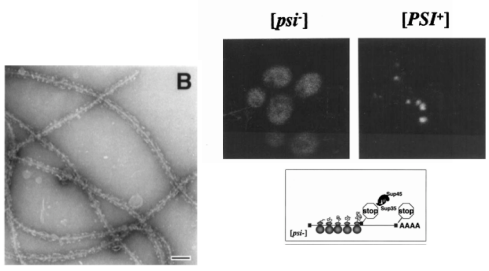


Weird genetics of read-through

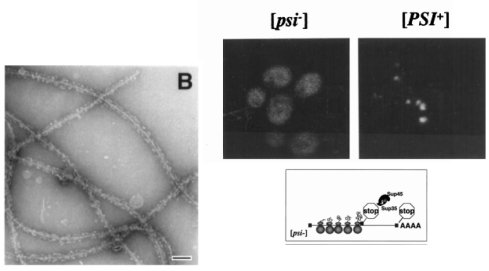


But why wouldn't it get diluted over many divisions?

Cytoplasmic aggregates of Sup35

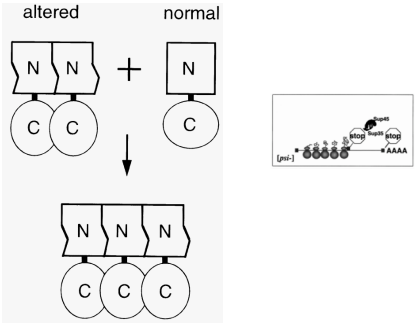


Cytoplasmic aggregates of Sup35



How would this explain the results?

Hard to make aggregate, easy to join



Protein inheritance

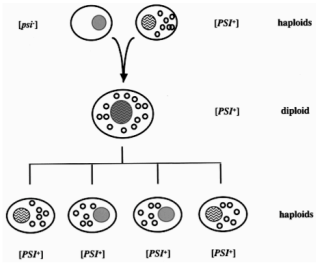
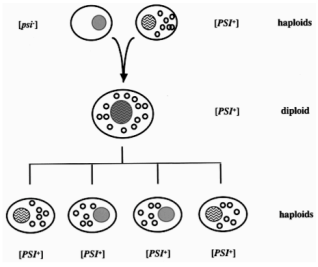


FIG. 1. [*PSI*$^+$] is cytoplasmically inherited. Black circles: [*PSI*$^+$] determinant. Yeast cells can grow either as haploids (one copy of each chromosome) or diploids (two copies of each chromosome). When a [*PSI*$^+$] haploid is mated to a [*psi*$^-$] haploid, the resulting diploid is [*PSI*$^+$], indicating that [*PSI*$^+$] is dominant. On sporulation, each nuclear determinant (gray fill and wavy lines) segregates to half the haploid progeny (2:2), and the cytoplasmic [*PSI*$^+$] determinant segregates to all progeny (4:0).

Protein inheritance



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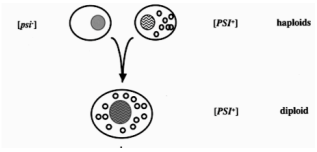
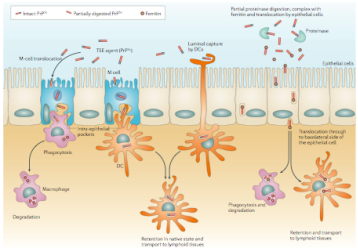
Original from *PSI+* nucleates in all progeny

PSI+ begets more *PSI+*



Not due to dominant genetics in the usual way.

Nucleating prions in mad cow disease



Prions and their lethal journey to the brain

Neil A. Mabbott* and G. Gordon MacPherson†

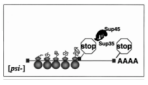
Is *PSI+* the yeast analog of mad cow or Alzheimer's?

If bad, would be lost

```
Sup35NMPm  1-  MSQDQQQQQQFNANNLAGNVQNINLNAPAYDPAVQSYIPNTAQAFVPSAQPYIPGQQEQQ -60
Sup35NMSc  1-  MSDSNQGNNQQNYQQYSQNGNQQQGNNR-YQGY-QAYN---AQAQ-P-AGGYY-----QN -48

Pm  61-  FGQYGQQQQNYNQGGYNNYNNRGGYNNNRGGYNNSNRGGYSNYNSNQGGYSNYNN -120
Sc  49-  YQGYSG----YQQGGYQQYNPDAGYQQQ---YNP---QGGYQQYNP------QGGY---QQ -90

Pm  121-  NYANNSYNNNNNYNNNYNQGYNNYNSQPQGDQQQETGSGQMSLEDYQKQQKESLNKLNT -180
Sc  91-   QFNPQGGRG--NYKNF-N--YNN-NLQGY-QAGFQPQSQG-MSLNDFQKQQKQA----AP -138

Pm  181-  KPKEVLKLNLNSSTVKAPIVTKKKEKEEPVNQESKTEEPAKEEIKNQEPAKAENKVEEESK -240
Sc  139-  KPKETLKL-VSSSGIKLANATKKVGTKPAESDKKEEEKS-AETK--EPTKEPTKVEEPVK -192

Pm  241-  VEAPTAAKPVSESEFPASTPKTEAKASKEVAAAAAALKK-EVS-QAKKESNVTNADALVK -298
Sc  193-  KEE----KPV-QTE-----EKTEEK-S-ELPKVED-LKISE-STHNTNNANVTSADALIK -236

Pm  299-  EQEEQIDASIVNDM — Sup35C
Sc  236-  EQEEEVDDEVVNDM — Sup35C
```

Evolutionary conservation of prion-forming abilities of the yeast Sup35 protein

Yury O. Chernoff,* Alexey P. Galkin,† Eugene Lewitin, Tatiana A. Chernova,‡ Gary P. Newnam and Slava M. Belenkiy

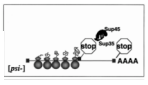


If bad, would be lost

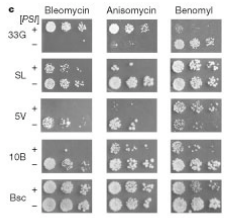
```
Sup35NMPm  1-  MSQDQQQQQQFNANNLAGNVQNINLNAPAYDPAVQSYIPNTAQAFVPSAQPYIPGQQEQQ -60
Sup35NMSc  1-  MSDSNQGNNQQNYQQYSQNGNQQQGNNR-YQGY-QAYN---AQAQ-P-AGGYY-----QN -48

(Pichia methanolica vs. S. cerevisiae)

Pm  61-  FGQYGQQQQNYNQGGYNNYNNRGGYSNNRGGYSNYNSYNTNSNQGGYSNYNN -120
Sc  49-  YQGYSG----YQQGGYQQYNPDAGYQQQ---YNP---QGGYQQYNP------QGGY---QQ -90

Pm  121-  NYANNSYNNNNNYNNNYNQGYNNYNSQPQGDQQQETGSGQMSLEDYQKQQKESLNKLNT -180
Sc  91-   QFNPQGGRG--NYKNF-N--YNN-NLQGY-QAGFQPQSQG-MSLNDFQKQQKQA----AP -138

Pm  181-  KPKEVLKLNLNSSTVKAPIVTKKKEKEEPVNQESKTEEPAKEEIKNQEPAKAENKVEEESK -240
Sc  139-  KPKETLKL-VSSSGIKLANATKKVGTKPAESDKKEEEKS-AETK--EPTKEPTKVEEPVK -192

Pm  241-  VEAPTAAKPVSESEFPASTPKTEAKASKEVAAAAAALKK-EVS-QAKKESNVTNADALVK -298
Sc  193-  KEE----KPV-QTE-----EKTEEK-S-ELPKVED-LKISE-STHNTNNANVTSADALIK -236

Pm  299-  EQEEQIDASIVNDM — Sup35C
Sc  236-  EQEEEVDDEVVNDM — Sup35C
```

Evolutionary conservation of prion-forming abilities of the yeast Sup35 protein

Yury O. Chernoff,* Alexey P. Galkin,† Eugene Lewitin, Tatiana A. Chernova,‡ Gary P. Newnam and Slava M. Belenkiy

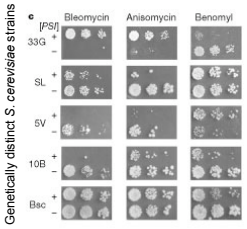


PSI+ phenotypes



A yeast prion provides a mechanism for genetic variation and phenotypic diversity

PSI+ phenotypes



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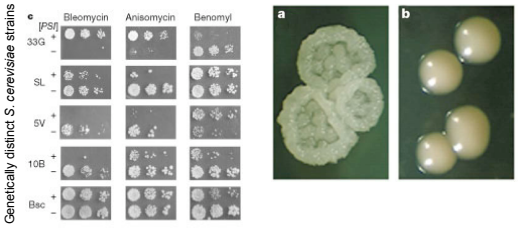

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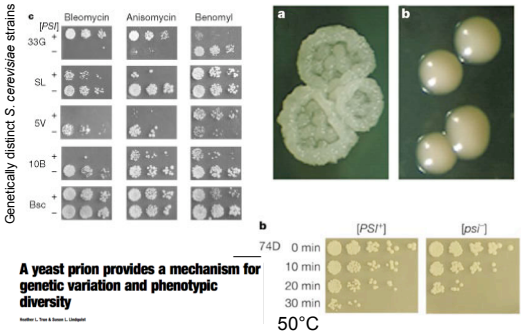

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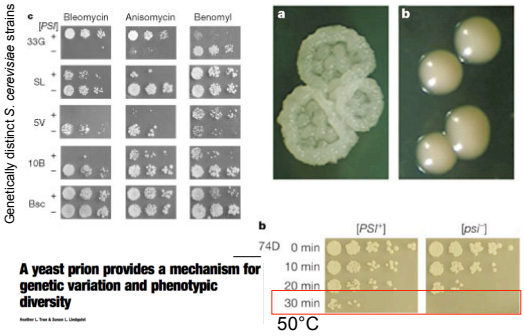


A yeast prion provides a mechanism for genetic variation and phenotypic diversity

PSI+ phenotypes

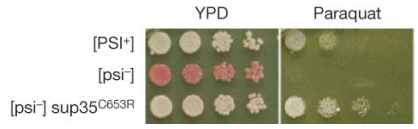
Why would aggregates result in so many novel abilities and traits??

PSI+ phenotypes

Why would aggregates result in so many novel abilities and traits??

Apparently does not happen in Alzheimer's…

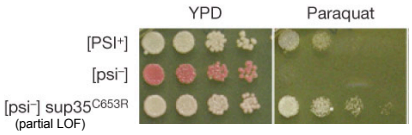
The clincher



Epigenetic regulation of translation reveals hidden genetic variation to produce complex traits

Heather L. True*, Rana Berlin & Susan L. Lindquist

The clincher



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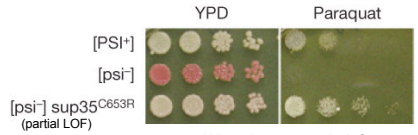

What do you conclude?

A. Sup35 does not cause resistance to paraquat.
B. Prions do not cause resistance to paraquat.
C. Aggregation is not necessary to cause resistance to paraquat.
D. Aggregation is not sufficient to cause resistance to paraquat.

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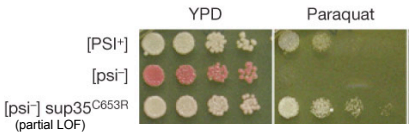
The clincher



Aggregates per se don't cause resistance.

Epigenetic regulation of translation reveals hidden genetic variation to produce complex traits

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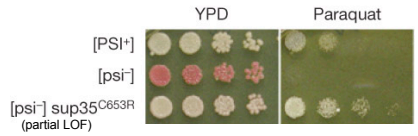


Aggregates per se don't cause resistance.
Losing function of sup35 does.

Epigenetic regulation of translation reveals hidden genetic variation to produce complex traits

Heather L. True*, Rana Berlin & Susan L. Lindquist

Aggregation = more read-through

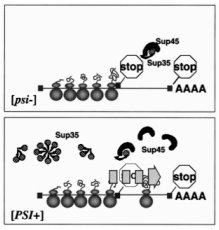
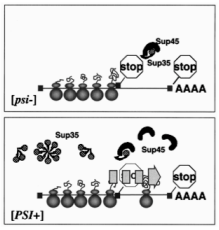


FIG. 3. Model for the [*PSI*+] phenotype. The black line denotes a messenger RNA with a stop codon mutation in the coding sequence. In [*psi*-] cells (top), Sup35 (hatched spheres with black, crescent tails) is soluble and binds to Sup45 (black crescent). The Sup35/Sup45 complex promotes translation termination at all nonsense codons (stop). In [*PSI*+] cells (bottom), Sup35 forms aggregates in the cytoplasm through interaction of the N-terminal domain (black crescent). The level of soluble Sup35 available for binding to Sup45 is greatly reduced. Consequently, ribosomes (gray spheres) are able to read through nonsense codons at a low frequency (dashed arrow), leading to the production of full-length protein, which suppresses the effects of the mutation.

Aggregation = more read-through



New extra-long forms of proteins cause new traits.

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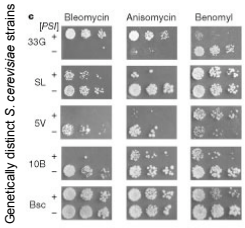
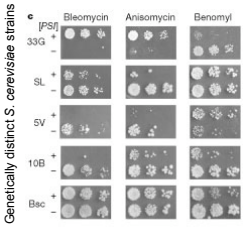
PSI+ allows epigenetic change in protein sequence.

Genetic effects?



Phenotypes varied between genetically diverse *PSI*+ strains.

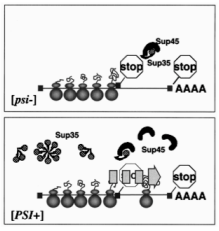
Genetic effects?



Phenotypes varied between genetically diverse *PSI*+ strains.

How can the cause be genetic and non-genetic (protein aggregates) at the same time?

Aggregation = more read-through



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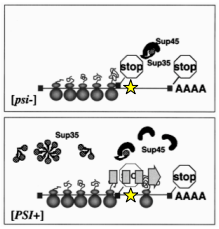

UTR mutations usually occur and have no effect

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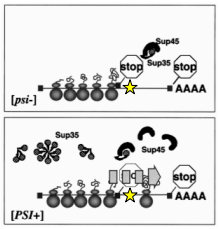

UTR mutations usually occur and have no effect, so organism doesn't die and allele is maintained.

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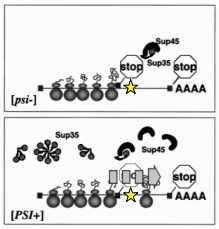

UTR mutations usually occur and have no effect, so organism doesn't die and allele is maintained.
Now in *PSI+*, they manifest!

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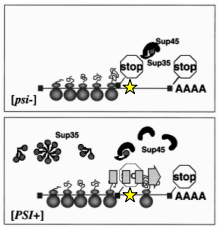

Sequence differences in read-through region cause phenotypic differences between *PSI+* strains.

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Cryptic variation: DNA sequence differences between individuals that are usually not expressed