CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Edith Heard

Année 2012-2013 :
"Épigénétique, développement et hérédité"

11 Mars, 2013

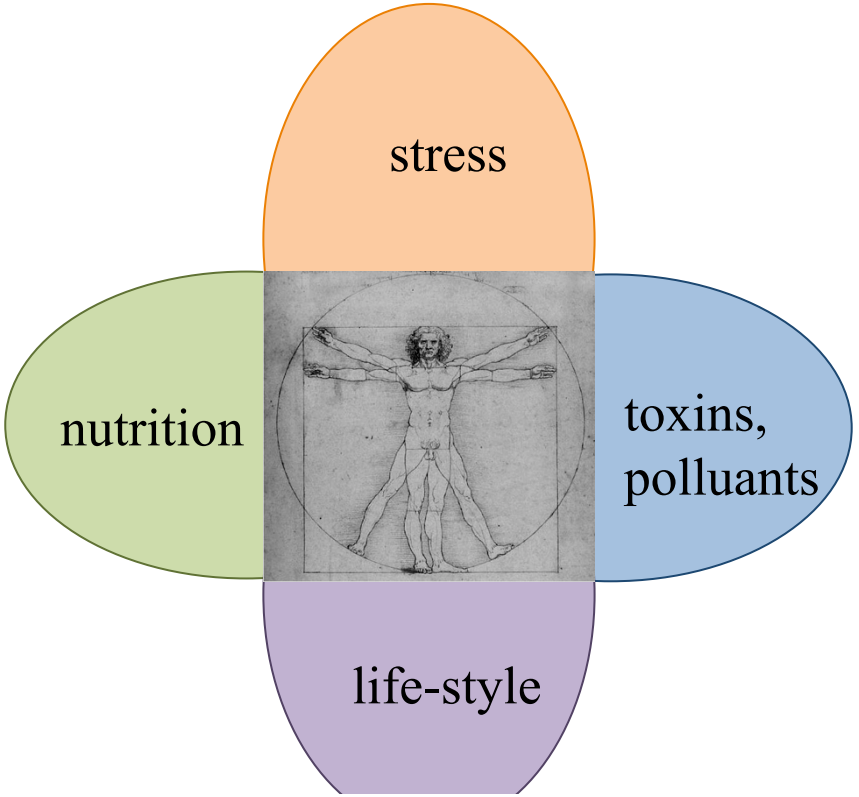
Cours V
"Mémoire de l'environnement"

Séminaire
Vincent Colot
(IBENS)

Epigenetics and the Environment

How much influence does the environment exert on epigenetic processes?

What is the impact on our epigenomes of:
Climate, Nutrition
Toxins, Stress, Aging



Gustav Klimt
The three ages of a woman
(1905)

Epigenetics and the Environment

How much influence does the environment exert on epigenetic processes?

What is the impact on our epigenomes of:
Climate, Nutrition
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How much of this is epigenetic (heritable) and how much is transient?

What *drives* epigenetic changes at some genes?

How do epigenetically altered states influence phenotype (development, physiology, behaviour, disease…)? How can they be reversed?

How does stress and the environment impact on repeats?

Can epigenetic marks can be perpetuated across generations? How? Why…

Gustav Klimt
The three ages of a woman
(1905)

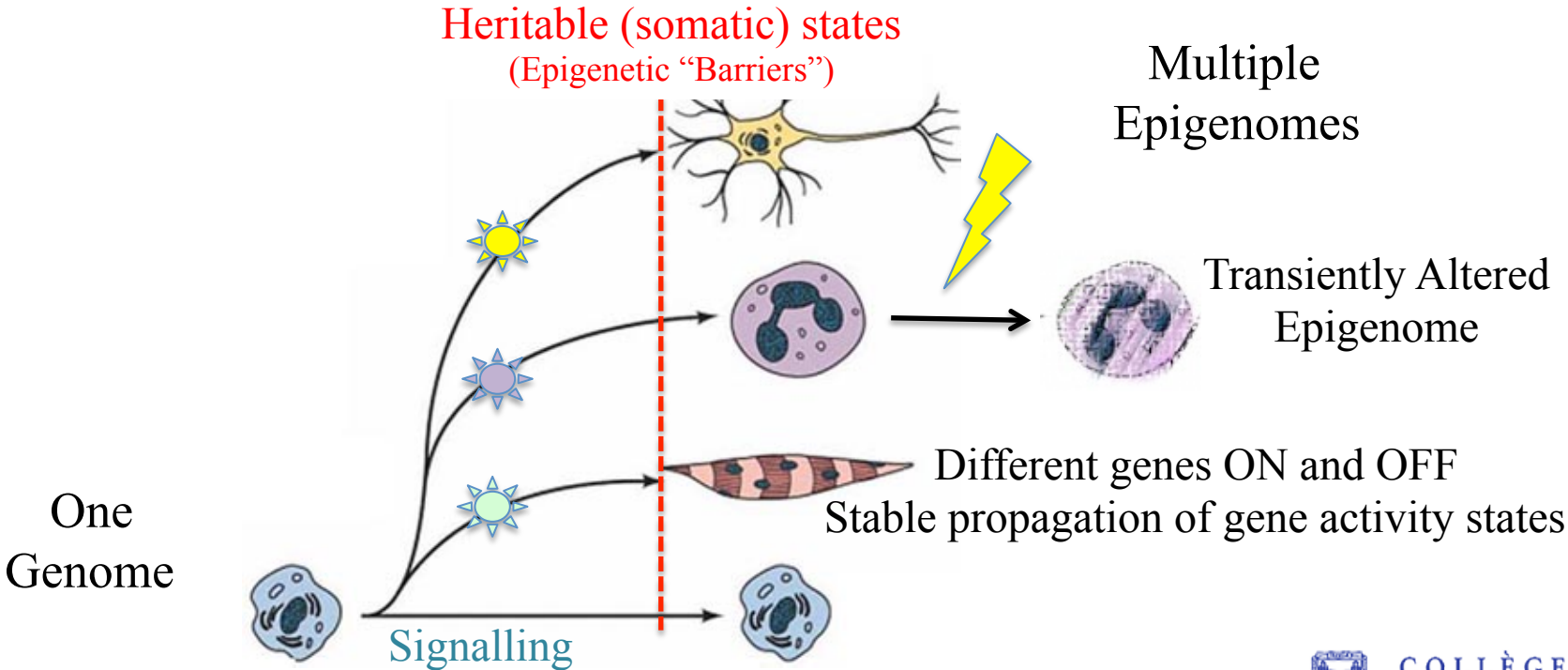
Today's seminar & lecture next week

Epigenetics and Epigenomics

Epigenetics: changes in gene expression or genome function that are stable over rounds of cell proliferantion or across generations but do not involve changes in the primary sequence of the cell/organism

Epigenomics: analysis of chromatin, RNA, and other "epigenetic" changes across many genes in cells of an entire organism

Some **epigenomic** changes can be heritable eg differentiation states, X-chromosome inactivation, imprinting, … but <u>many</u> are <u>not.</u>



(Eg internal signals, positional information, cell-cell contacts, growth factors, etc
Essential to establish cell type, patterning, morphogenesis)

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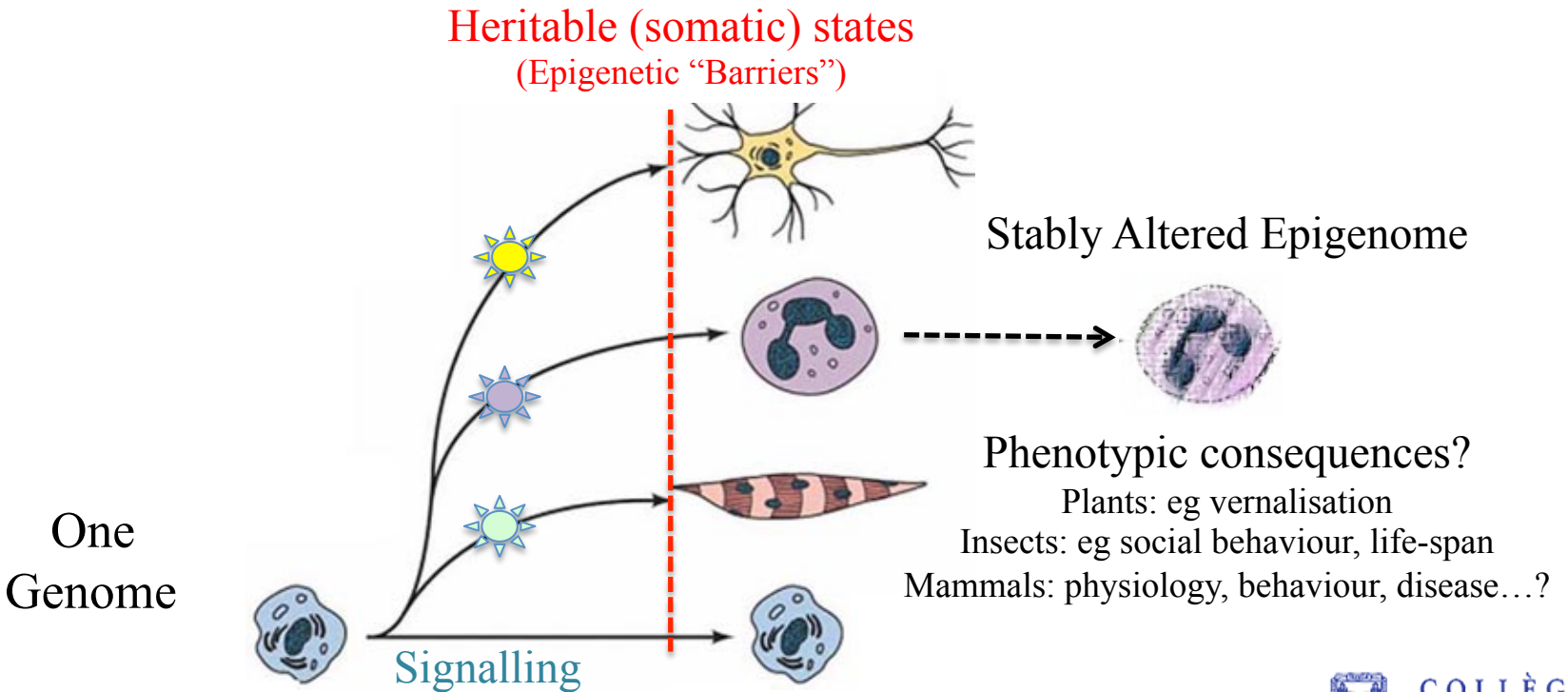

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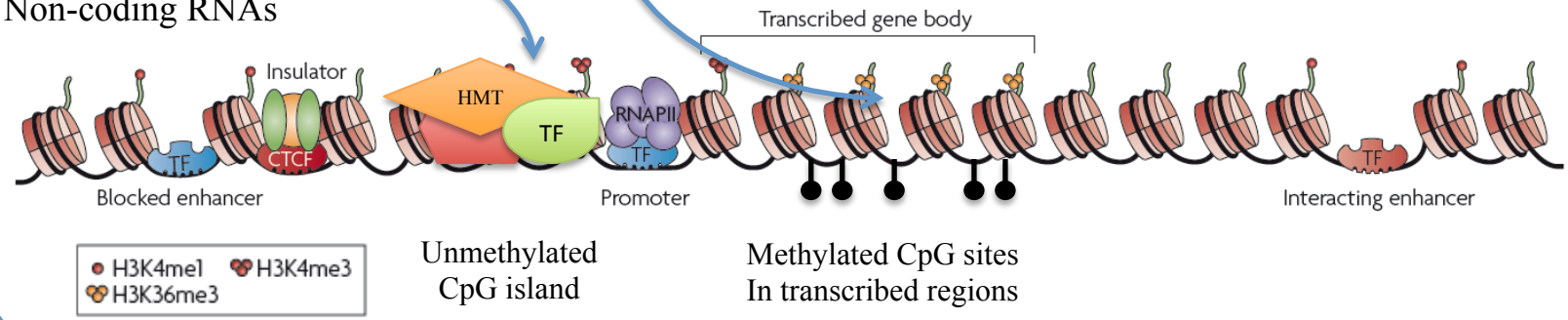
Inducing Changes in Epigenetic States

Differentiation Signals

NB Although chromatin marks and DNA methylation appear at specific regions of the genome – the enzymes that lay them down (HMTases, DNMTs etc) have no sequence specificity & rely on DNA binding factors (eg TFs), or non-coding RNAs, for their recruitment.

Initiators:
DNA binding factors
Non-coding RNAs



Unmethylated
CpG island

Methylated CpG sites
In transcribed regions

Changes in
Some histone modifications,
Histone variants
PcG or Trx group protein binding
DNA methylation

MAINTENANCE?

Adapted from B. Ren, NRG, 2010

Inducing Changes in Epigenetic States

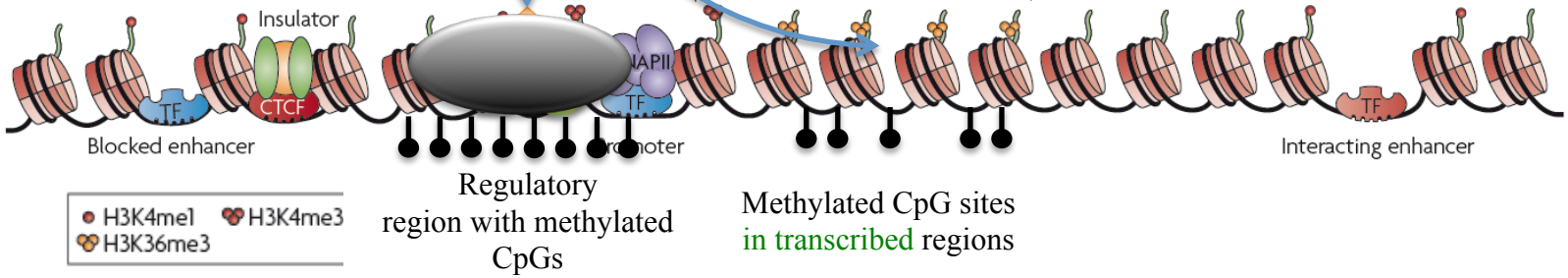
ENVIROMENTAL EVENTS?
Temperature changes
Nutritional signals etc.

STOCHASTIC EVENTS?

METASTABLE STATES?

???

Initiators:
DNA binding factors
Non-coding RNAs



Regulatory
region with methylated
CpGs

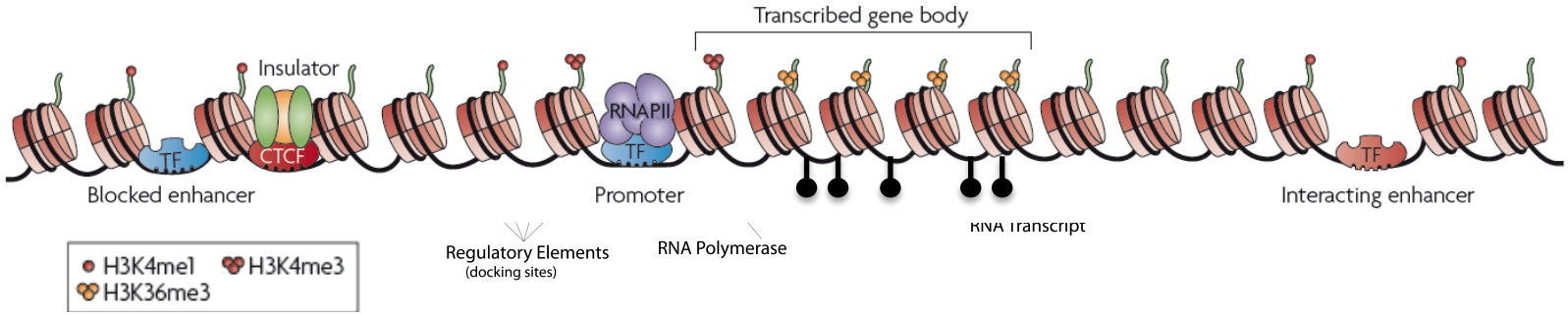
Methylated CpG sites
in transcribed regions

Changes in
Some histone modifications,
Histone variants
PcG or Trx group protein binding
DNA methylation

MAINTENANCE?

Adapted from B. Ren, NRG, 2010

Changes in Epigenetic and Epigenomic States



The stability of chromatin changes can vary: some may be transient, others are longer lasting. Some chromatin changes are mitotically heritable and can affect somatic tissues, whereas others may even be inherited through meiosis and affect the next generation.

Epigenetic states may also be influenced by genetic variation (polymorphisms or mutations in genes and regulatory sequences), but the extent to which this is the case is unknown.

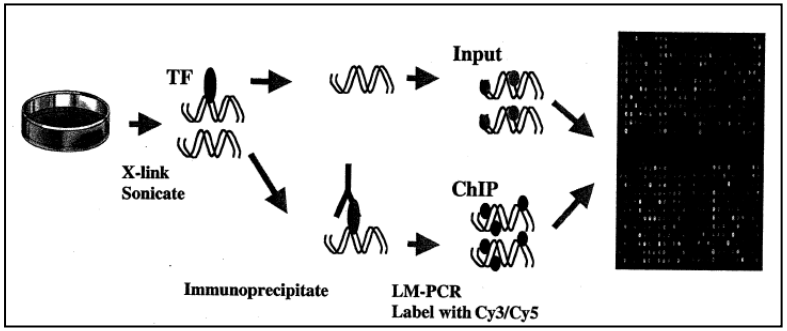
⇒ Potentially an extremely large number of possible epigenomes that could be mapped. Challenge: although all cells of an organism have essentially the same genome – each tissue can consist of mixed cell types/states & **multiple epigenomes** - a single organism may have thousands of epigenomes.

The ability to collect epigenomic data at high resolution enables investigations of the changes that occur due to development, lineage and tissue-specificity - as well as those caused by environmental influence, such as ageing, stress, diet, hormones or toxins.

Epigenomic Mapping

1. Chromatin mapping:

   ChIP chip, ChIP-Seq, meDIP-Seq (Chromatin immunoprecipitation followed by hybridisation to microarrays or sequencing)



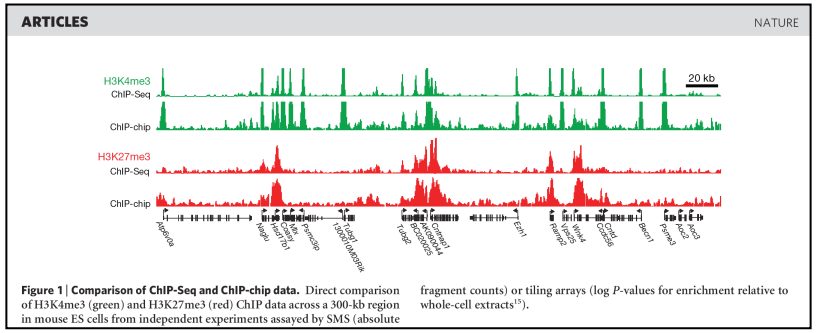
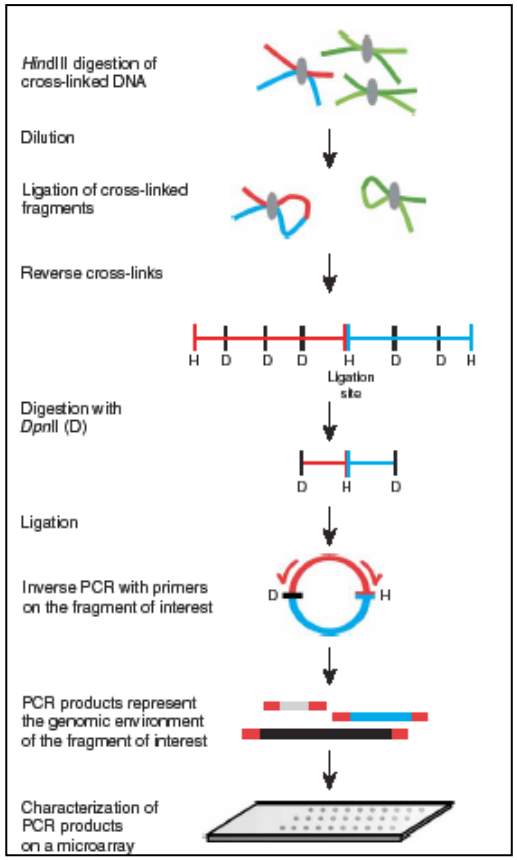


Figure 1 | Comparison of ChIP-Seq and ChIP-chip data. Direct comparison of H3K4me3 (green) and H3K27me3 (red) ChIP data across a 300-kb region in mouse ES cells from independent experiments assayed by SMS (absolute fragment counts) or tiling arrays (log *P*-values for enrichment relative to whole-cell extracts[15]).

2. Mapping of long range *cis* and *trans* interactions in the nucleus :

   Chromosome conformation capture technologies



3. Transcription and RNA

   Transcriptome (RNA-Seq), transcriptional run-ons (GRO Seq), small RNA analysis sRNA-Seq (miRNA, siRNA, piRNA…)

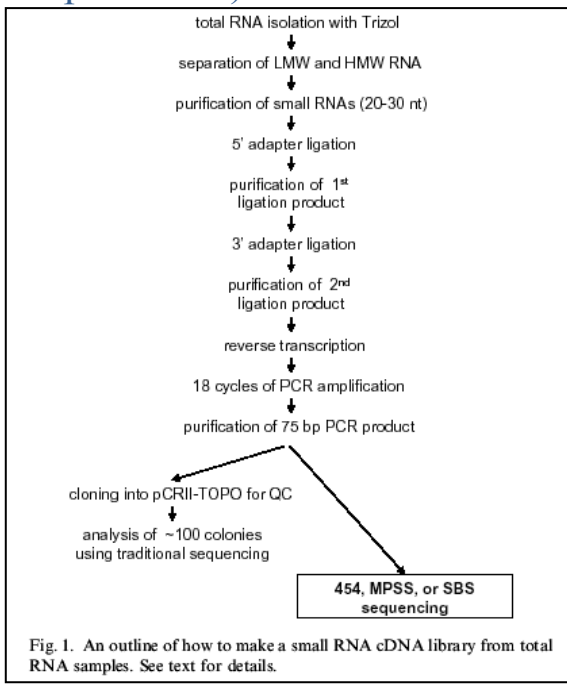
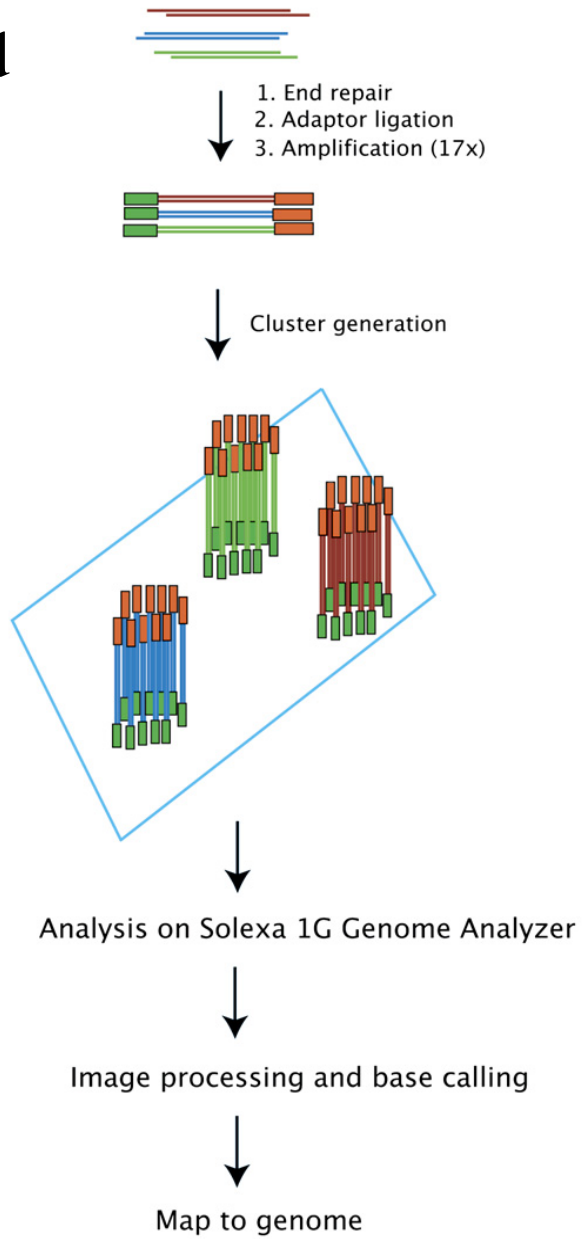


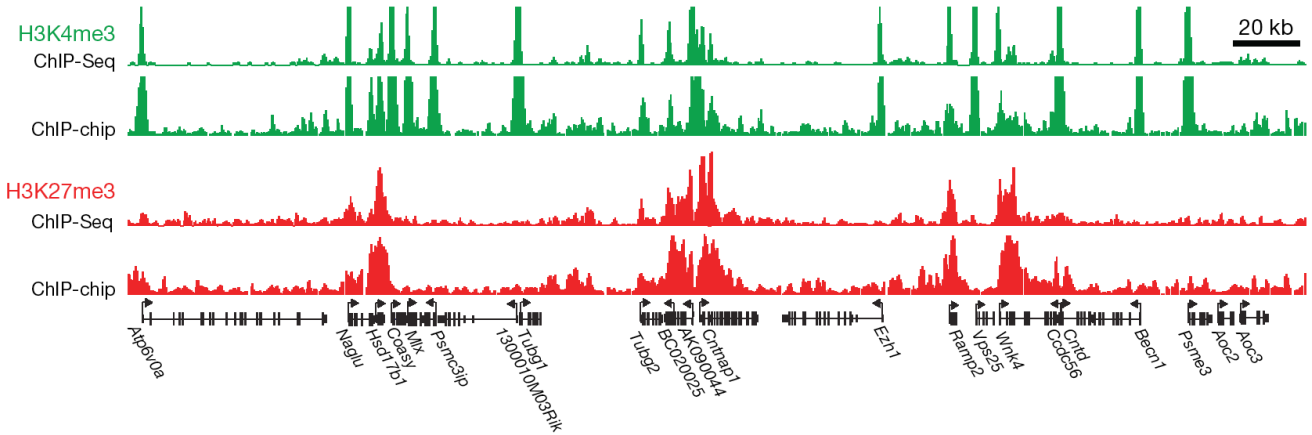
Fig. 1. An outline of how to make a small RNA cDNA library from total RNA samples. See text for details.

Epigenomic Mapping

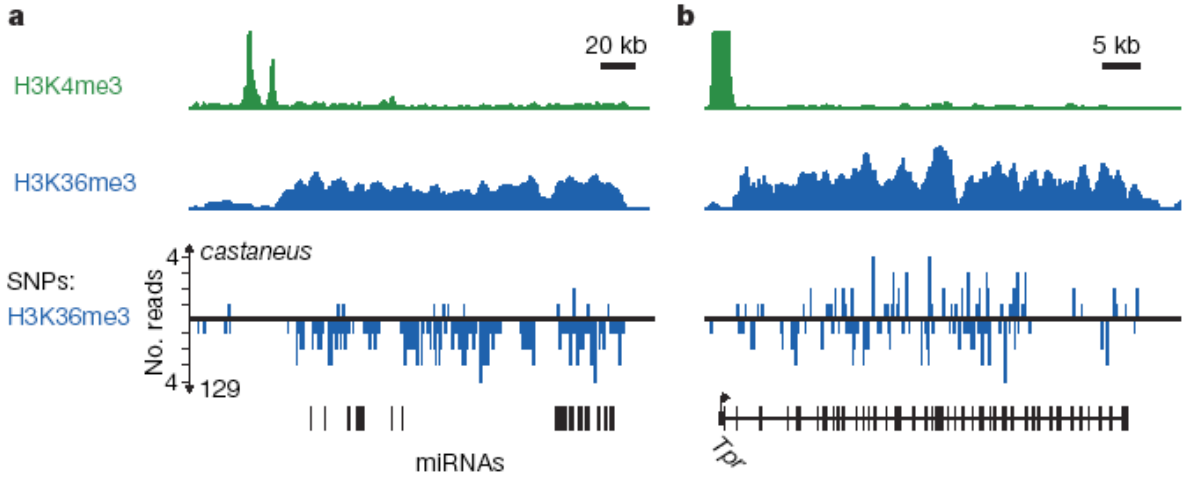
Reading the genome and the epigenome using next generation sequencing (NGS) technology …



Epigenomic Landscapes



Allele-Specific Epigenomic Marks



Imprinted expression
(129 allele only)

Non-imprinted expression
(Cast and 129 alleles)

From Mikkelsen et al, Nature, 2007

The Power of Epigenomics

Genome Annotation: chromatin signatures enable efficient and precise genome annotation of *regulatory elements* (to which DNA sequence-specific factors can bind), and can pinpoint functional or cell type-specific regions of interest (see "ENCODE" – Nature 489, September 2012)

Allele-Specific mapping: distinction of the states of the two alleles, when sequence polymorphisms are available

Cell identity: While genes are either expressed or not, chromatin states can add further refinement to a gene's activity status, eg whether it is primed or poised, and can also describe varying degrees of repressed states that would all look the same by any gene expression measure. The precise chromatin state of these loci can have clear consequences for how they behave in both normal development and disease.

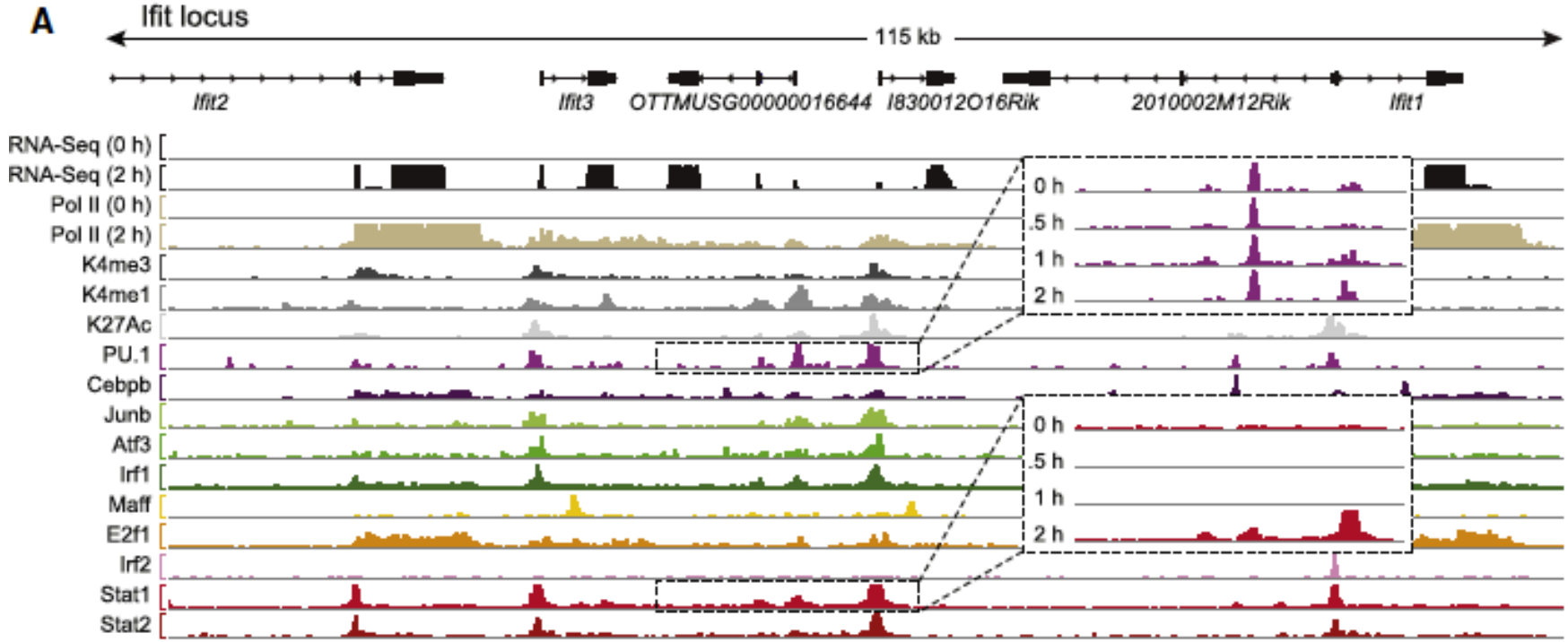
Developmental Dynamics and Disease: Epigenomic maps can be utilized to trace the origin of cells, dissect effected pathways and identify predictive biomarkers. Epigenome data can also help pinpoint disease-relevant regulatory elements through epigenome-wide association studies, or 'EWAS', especially when integrated with data from genome-wide association studies (GWAS).

See Meissner *Genome Biology* 2012, **13**:420

Dynamic Epigenomic Landscapes

Gene expression and epigenomic dynamics

Epigenomic landscape of dendritic cells at four different time-points after a pathogenic stimulus:



- Overlaying ChIP-seq data on gene-expression dynamics reveals that many TF/DNA interactions are established *prior* to the stimuli
- "Pioneer" factors potentiate binding by opening previously inaccessible sites
- "Primer" TFs (eg Jun-b) prime the response and set basal expression levels of many genes
- Other TFs dynamically bind subsets of genes of a shared biological process

Garber et al (2012) "A High-Throughput Chromatin Immunoprecipitation Approach Reveals Principles of Dynamic Gene Regulation in Mammals" Mol. Cell 47, 810–822

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Dynamic Epigenomic Landscapes

Epigenomic landscapes are being used to explore the changes that are induced under different environmental conditions, the positions and nature of these changes, their extent and longevity….

ENVIRONMENTALLY PROGRAMMED EPIGENETIC CHANGES

1. Social insects represent a unique model for how the same genome can give rise to entirely different phenotypes — soldiers, common labourers, and queens.

2. Plants provide remarkable examples of climate-controlled programming - eg cold-induced flowering time (vernalisation)

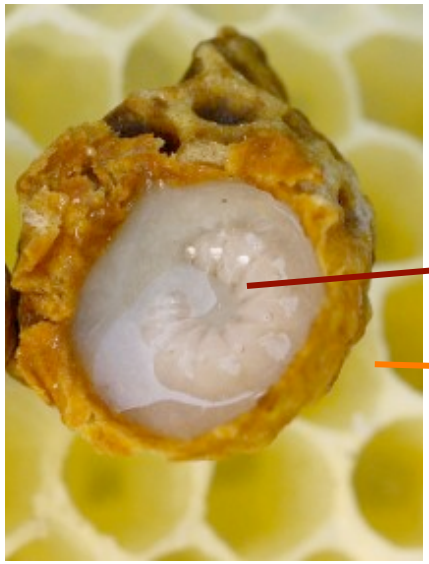
Nutrition, Phenotype and Epigenotype in the Honeybee

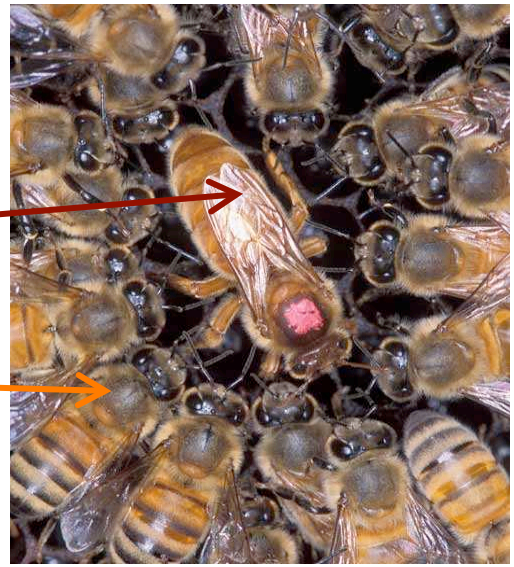
One genome – two destinies…

• A female bee's place in the social order—whether she becomes a *worker* or a *queen*—depends not just on her genes, but on whether she eats royal jelly.

• Nutrition and juvenile hormone (JH) signaling determine the caste fate of the individual bee.

• Larval nutrition on royal jelly results in queen formation – sister larvae, fed briefly on royal jelly and then on pollen and nectar, become workers – despite SAME genetic constitution.





Queen

Life-span: 3/4 years

Roles: reproduction, social structure of the hive

Worker

Life-span: 40 days to 6 months

Functions : Nursing, cleaning, building , foraging

Nutrition, Phenotype and Epigenotype in the Honeybee

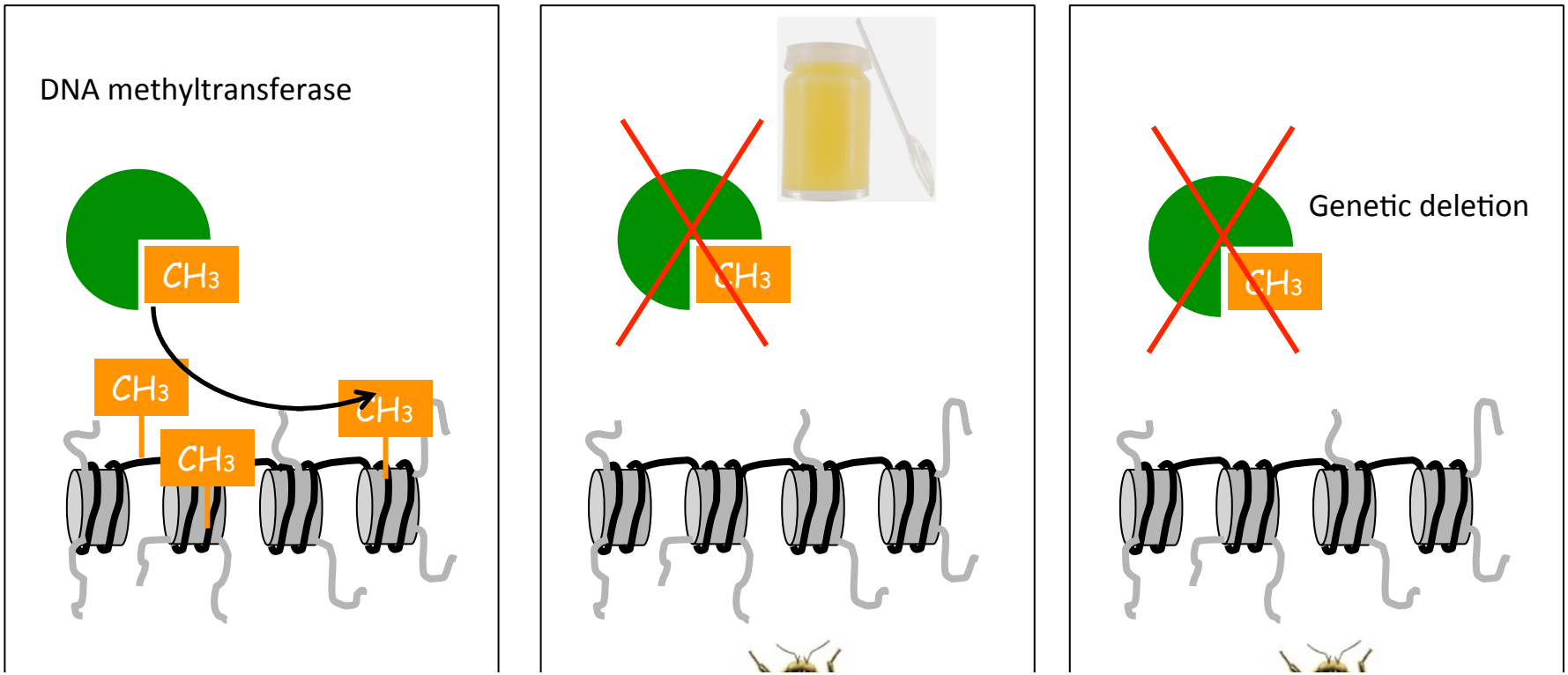
Workers are not just a reproductively repressed form of queens.
They have a highly differentiated behavioral repertoires with functions *not* displayed by queens - workers are active and intelligent skillfully navigating the outside world in search for food for the colony

	Worker	Queen
Mass at emergence	81–151mg	178–292mg
Development egg->adult	16–24 days	14–17 days
Age	15–38 days (summer bees) 140 days (winter bees)	1–3 years normally (up to 8 yrs in some cases)
Facets in compound eye	5,000–6,000	3,500
Placoid olfactory sensillae	2,700	1,600
Pollen basket	Yes	No
Wax glands	Yes	No
Spermatheca	Rudimentary	Large
Ovarioles	2–12	150–180
Sting barbs	Yes	Rudimentary
Mandibular glands	Large	Very large
Nasonov glands	Yes	No
Dance communication	Yes	No

What are the differential gene expression patterns and epigenetic changes that account for differences triggered by differential nutrition?

- Proteins in royal jelly (such as royalactin) are responsible for many of the physical differences that distinguish queens from workers – and can even induce queen bee-like traits in fruit flies (Malezka et al, 2008)
- >1/5th of honeybee genes are differentially expressed between queens and workers (Weinstock et al, 2006; Grozinger et al, 2007)
- One component of royal jelly is phenyl buryrate (HDAC inhibitor) (Lyko et al, 2010)
- Silencing of DNMT3 in newly hatched larvae mimics effects of royal jelly – ie larvae destined to become workers develop into queens with fully developed ovaries (Kucharski et al, 2008)

Depleting DNMT3 (without Royal Jelly) produces a Queen



Accompanied by a transcriptional shift toward higher expression of physiometabolic genes, including genes coding for metabolic enzymes and general growth (TOR signalling)

Royal jelly provides the external information interpreted by the developing larva to create and maintain the epigenetic state necessary to generate a queen – and this involves DNA methylation?

How? Where? Which genes?

⇒ Epigenomic profiling…

E. He

Sequencing of the Honeybee Genome

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Honey Bee Epigenomics

The Honey Bee Epigenomes: Differential Methylation of Brain DNA in Queens and Workers

Frank Lyko[1,5], Sylvain Foret[2,5], Robert Kucharski[3], Stephan Wolf[4], Cassandra Falckenhayn[1], Ryszard Maleszka[3,*]

DNA methylation dynamics, metabolic fluxes, gene splicing, and alternative phenotypes in honey bees

Sylvain Foret[a,b], Robert Kucharski[b], Matteo Pellegrini[c], Suhua Feng[c], Steven E. Jacobsen[c,d,e,1], Gene E. Robinson[f], and Ryszard Maleszka[b,1]

Very different DNA Methylation distributions to plants and mammals:

Organism	Genome size (Mb)	Percentage of methylated cytosines in the CG context	Methylation status		
			Transposons and other repeats	Promoters, CpG islands[d]	Gene bodies
Arabidopsis thaliana	160	24[a]	Methylated	Methylated	Methylated
Homo sapiens	3300	70–80[b]	Methylated	Methylated	Methylated
Apis mellifera	260	0.7[c]	Not methylated	Not methylated	Methylated
Bombyx mori	530	<0.2	Not methylated	Not methylated	Methylated

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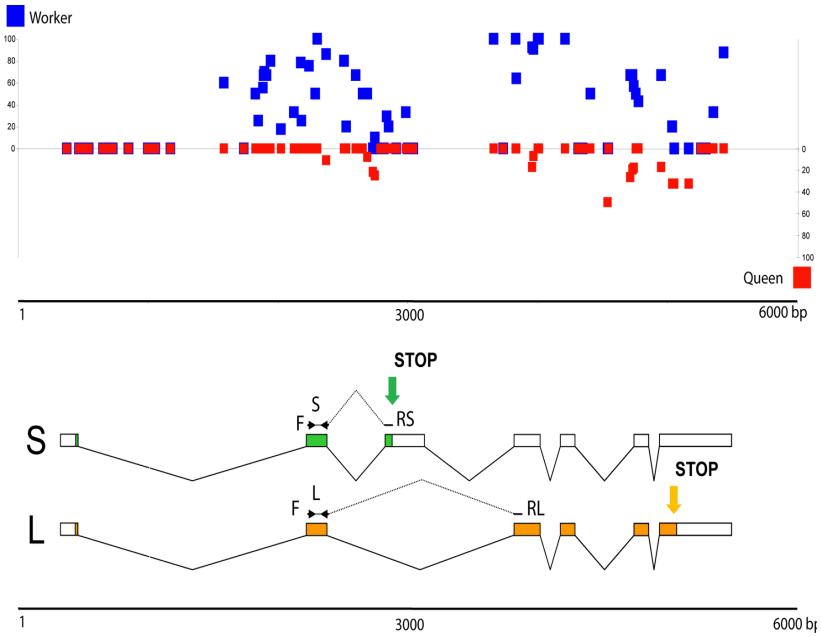
PLoS Biology, 8, e1000506 (2010)

CpG DNA methylation differences found:-

- in brains of workers and queen bees at >550 regions
- Mainly at conserved genes – many involved in brain development or activity
- Not at CpG islands and promoters, but in bodies of expressed genes – and correlating with splice variants that are differentially expressed in queens and workers...
- Genes encoding hormone receptors, and/or implicated in responses to juvenile hormone (JH) (eg TOR signaling), found to be differentially methylated



Conclusions:

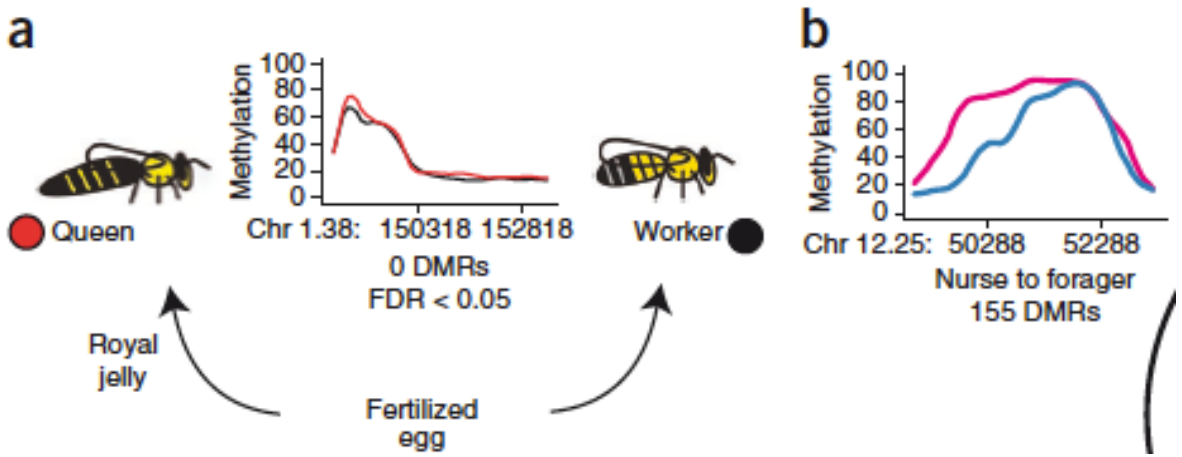
- DNA methylation differences do not correlate with gene repression but differential splicing
- Changes in expression of multiple genes – particularly involved in JH signalling - might act synergistically over many genes to achieve caste differences
- Whether DNA methylation differences are cause or consequence of such changes: still unclear

Honey Bee Epigenomics

Reversible switching between epigenetic states in honeybee behavioral subcastes

Brian R Herb[1,2,8], Florian Wolschin[3,4,8], Kasper D Hansen[1,5], Martin J Aryee[1,6], Ben Langmead[5], Rafael Irizarry[5], Gro V Amdam[3,4] & Andrew P Feinberg[1,2,5,7]

Nat.Neuro.,15, 1371-1374 (2012)



Open Questions

- Which genes are critical in queen / worker distinction and how do the chemical components of royal jelly trigger their differential expression?
- How/when does DNA methylation impact on expression profiles?
- What do changes in DNA methylation tell us about nurse/forager differential behaviours?

What are the signals? What are the genes?
What are the DNA binding factors that guide Dnmts & other epigenetic machineries to specific genes?

Specialization and Flexibility in Ant Castes

Camponotus floridanus: caste structure is rigid, queens and workers cannot swap functions - if the queen dies, the colony perishes

Harpegnathos saltator: queens and workers have similar morphology and life-span - workers can turn into functional queens (gamergates) if the need arises.

From Chittka et al, 2012, Current Biology, 22, R836

- Unlike honeybees, the trigger for worker/queen caste specialization not yet clear in Ants: Nutrition? Pheromones? Other…?
- Are more rigid caste-structured ants associated with more or less epigenetic marking…?

DNA Methylomes of different castes of two ant species:

• As in Honeybees, correlation exists between caste, alternative splicing and DNA methylation, which may (somehow) determine which exons are included in spliced transcripts of genes – and account for differential gene expression associated with caste.

• More DNA methylation differences between queens and workers in Camponotus

• Several differentially methylated genes associated with the queen–worker dimorphism are conserved between the two species.

Bonasio, R. et al. (2012) "Genome-wide and caste-specific DNA methylomes of the ants Camponotus floridanus and Harpegnathos saltator". *Curr. Biol.* 22, 1755–1764.

Temperature and Epigenetic changes in Plants

- **Short-term adaptation to temperature changes** in plants is partly mediated through a general mechanism that involves a histone variant histone H2AZ.

Nucleosomes containing H2AZ have DNA-unwrapping properties.
Cooler temperatures = increased H2AZ incorporation; Higher temperatures=decreased H2AZ incorporation
Differential histone-variant enrichment mediates levels of gene expression that are appropriate for the ambient temperature

- Many plants are completely dependent on subtle aspects of the weather to survive
Eg **Vernalization** – a period of cold required for appropriate flowering timing

NB Some of the plants that need to be vernalised are important food species such as sugar beet and wheat, which feed millions and provide much-needed income globally.

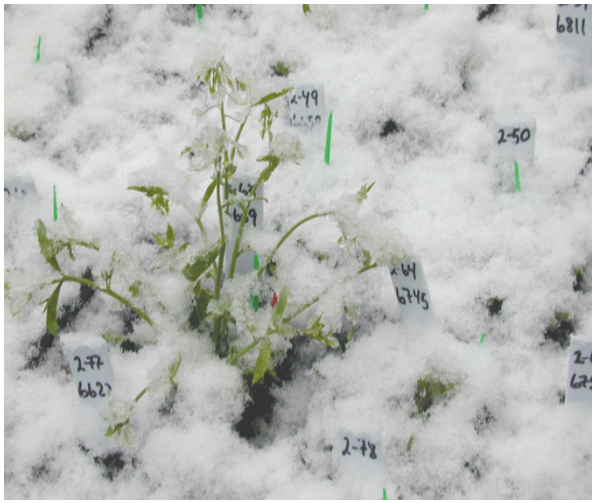
In nature, timing is crucial…

• Correct timing of flowering is key to reproductive success

• For example – to ensure that reproductive development and seed production occurs in spring and summer, rather than in autumn

• Multiple pathways have evolved to mediate different environmental and endogenous cues

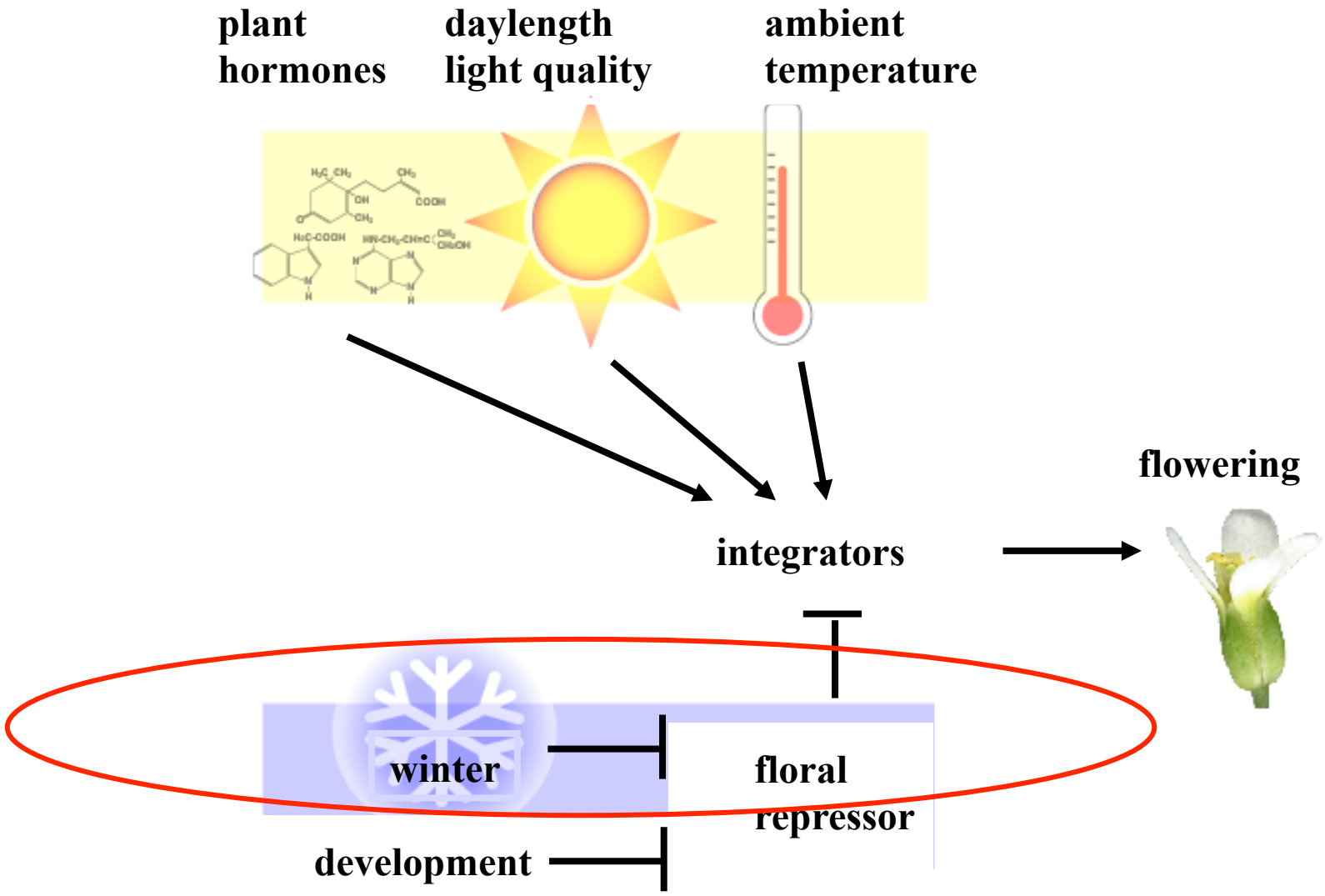
•For example, longer days as well as cold temperatures are required for winter wheat plants to go from the vegetative to the reproductive state (*VRN1, VRN2*, and *FT* (*VRN3*) genes)



J. Stinchcombe

Courtesy of C. Dean

Multiple environmental and endogenous cues regulate the switch to flowering

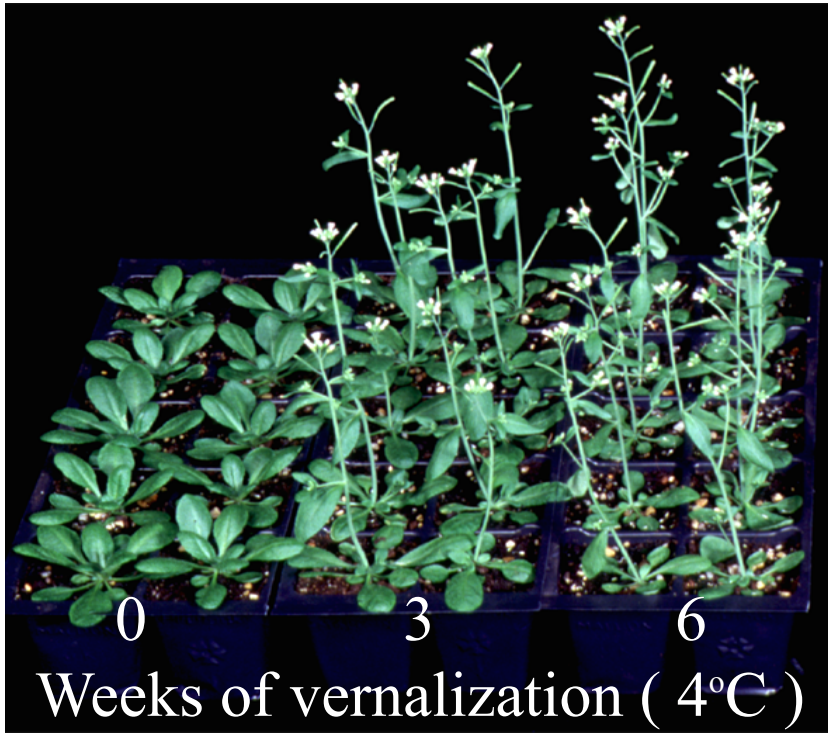


Courtesy of C. Dean

Vernalization

From Latin: vernus, of the spring

Acquisition of a plant's ability to flower in the spring by a chilling treatment (exposure to the prolonged cold of winter)



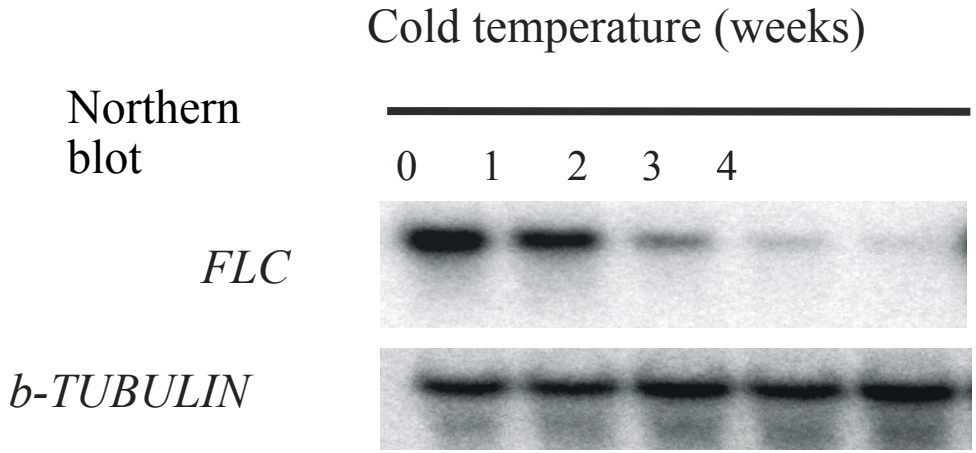
- quantitative
- reversible
- perceived by dividing cells
- not graft transmissible
- mitotically stable
- reset at meiosis

Heritable but reversible changes in gene expression …

After vernalization, plants have acquired ability to flower, but require additional seasonal cues or weeks of growth before they actually flower.

Vernalization

Vernalization causes a gradual reduction in expression of a floral repressor *FLC*



vernalization

⊥

FLC
(*Flowering Locus C = a Transcription Factor that represses the flowering program*)

⊥

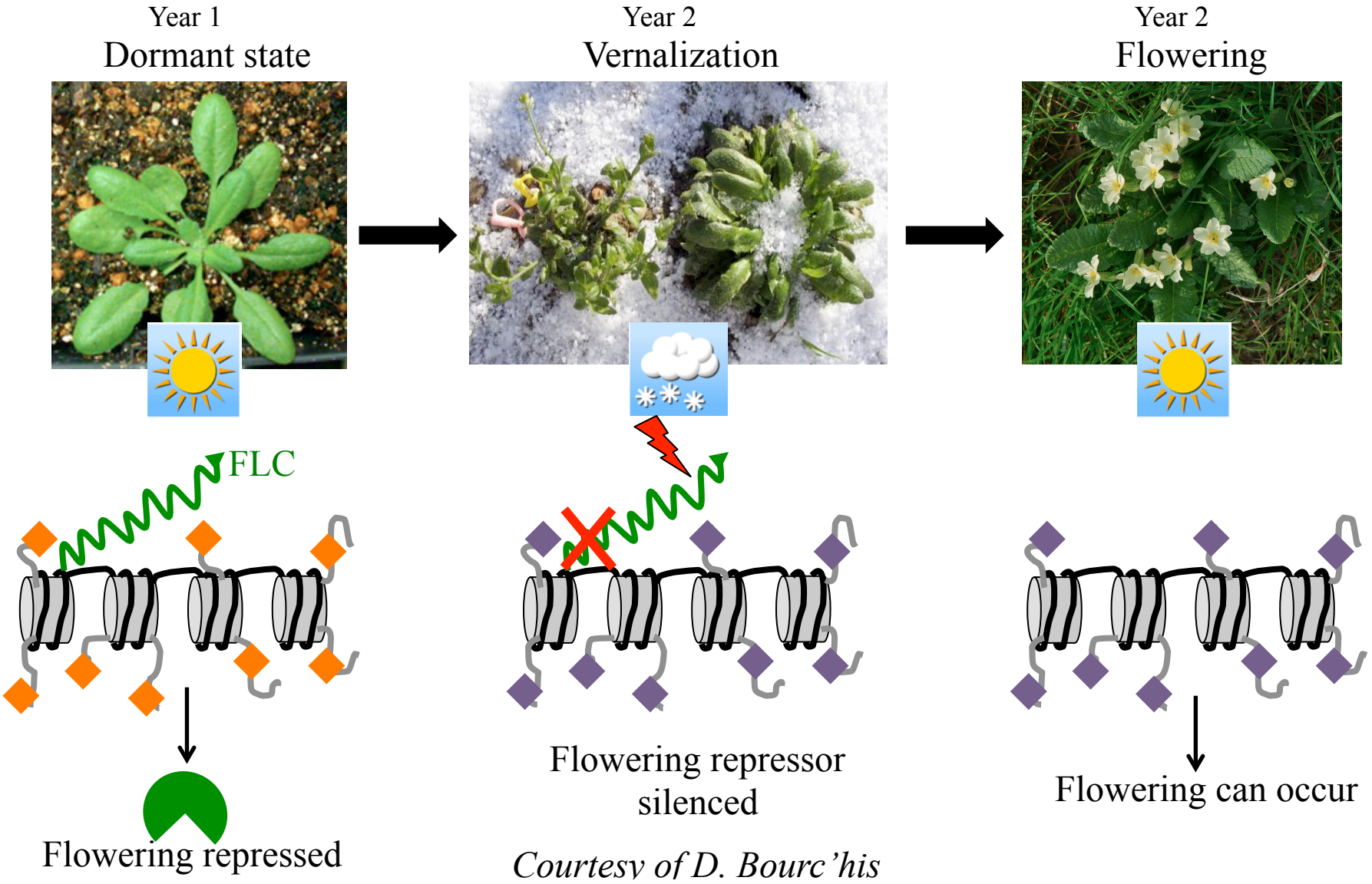
flowering

The expression then remains off throughout the rest of development

Courtesy of C. Dean

Vernalization

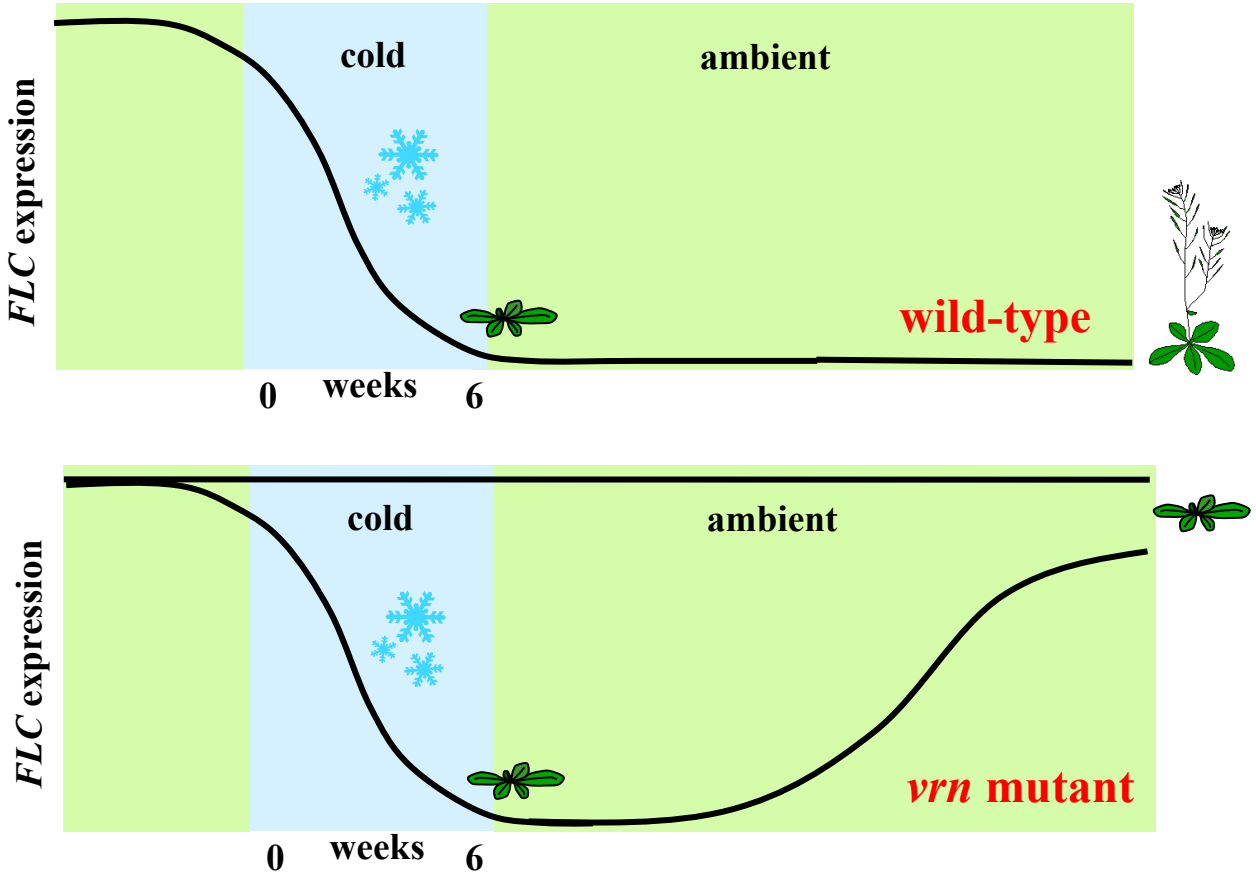
Vernalization causes a gradual reduction in expression of a floral repressor *FLC*



Courtesy of D. Bourc'his

Molecular Mechanisms ?

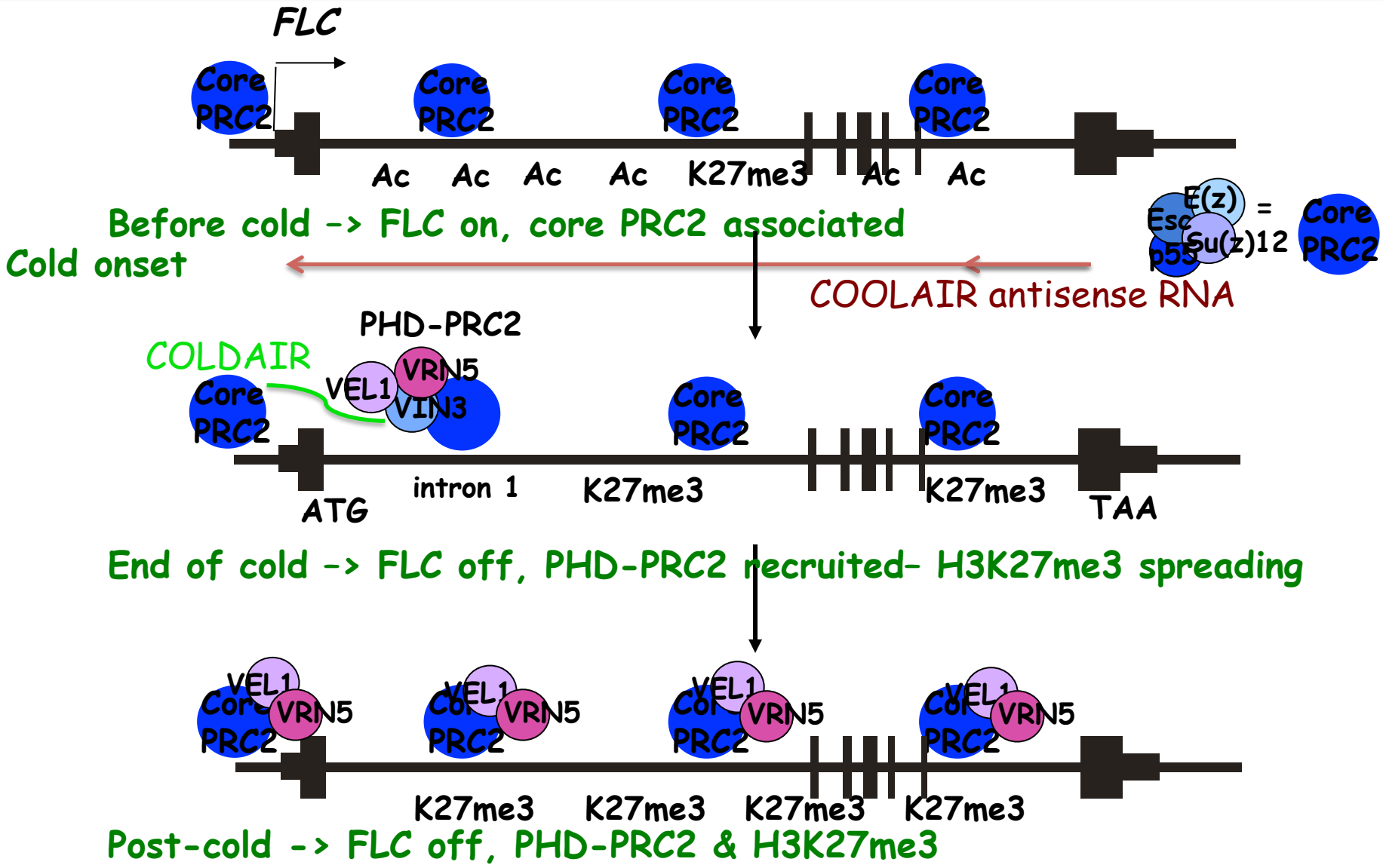
Forward genetic screen to identify components required for *FLC* silencing



Identify Polycomb group (Suz12) homologue, PHD proteins, DNA-binding protein, HP1 homologue

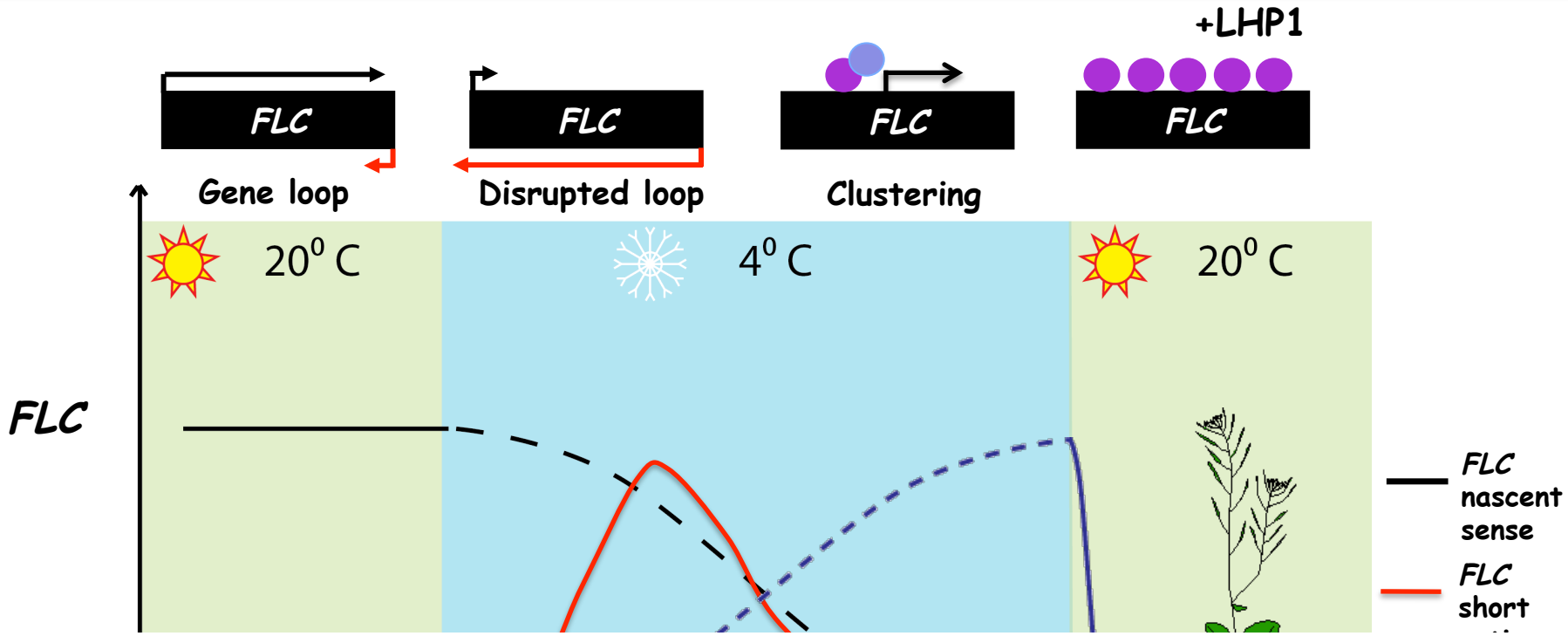
Courtesy of C. Dean

Polycomb group proteins and ncRNA



For review, see Letswaart et al (2012) *Trends Genet.* 28, 445–453

Different phases of the vernalization mechanism & adaptation



- Extensive natural variation in vernalization requirement and response in *Arabidopsis thaliana* accessions collected from different regions of the world.
- Molecular basis of this variation and its contribution to adaptation of Arabidopsis?
- Different aspects of this mechanism appear to have altered during *adaptation* of Arabidopsis in its different habitats

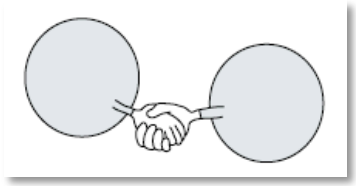
ENVIRONMENTALLY INDUCED EPIGENETIC CHANGES IN MAMMALS

What types of changes?
Where – Genes? Repeats?
What induces them?
Do they matter?
How transient are they?
Can they be considered as epigenetic (heritable)?

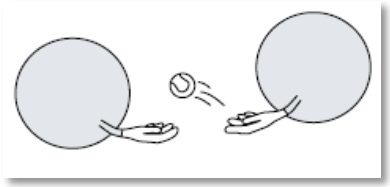
It still remains very poorly understood whether there are substantial epigenetic changes induced by the environment in mammals and whether these actually contribute to environmentally induced phenotypes….

Different environmental influences and epigenetic changes at different stages of life

Cell-cell communication

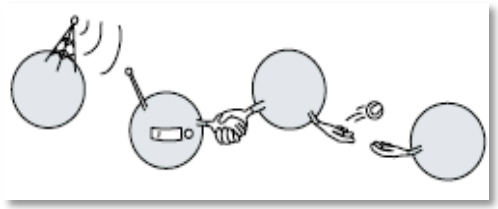


Chemical signalling (short range)



Chemical signalling (eg hormones) (long distance)

Envirnmental signals – all types

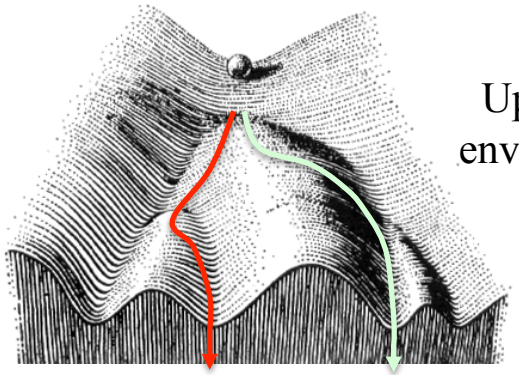


Courtesy of D. Bourc'his

Influence of envrionmental fluctuations during early mammalian development?

Mammalian epigenetic processes
(eg X inactivation, imprinting) appear to be remarkably resistant to environmental changes

Viviparous development occurs in highly protected environments
(in *utero*, germ line)

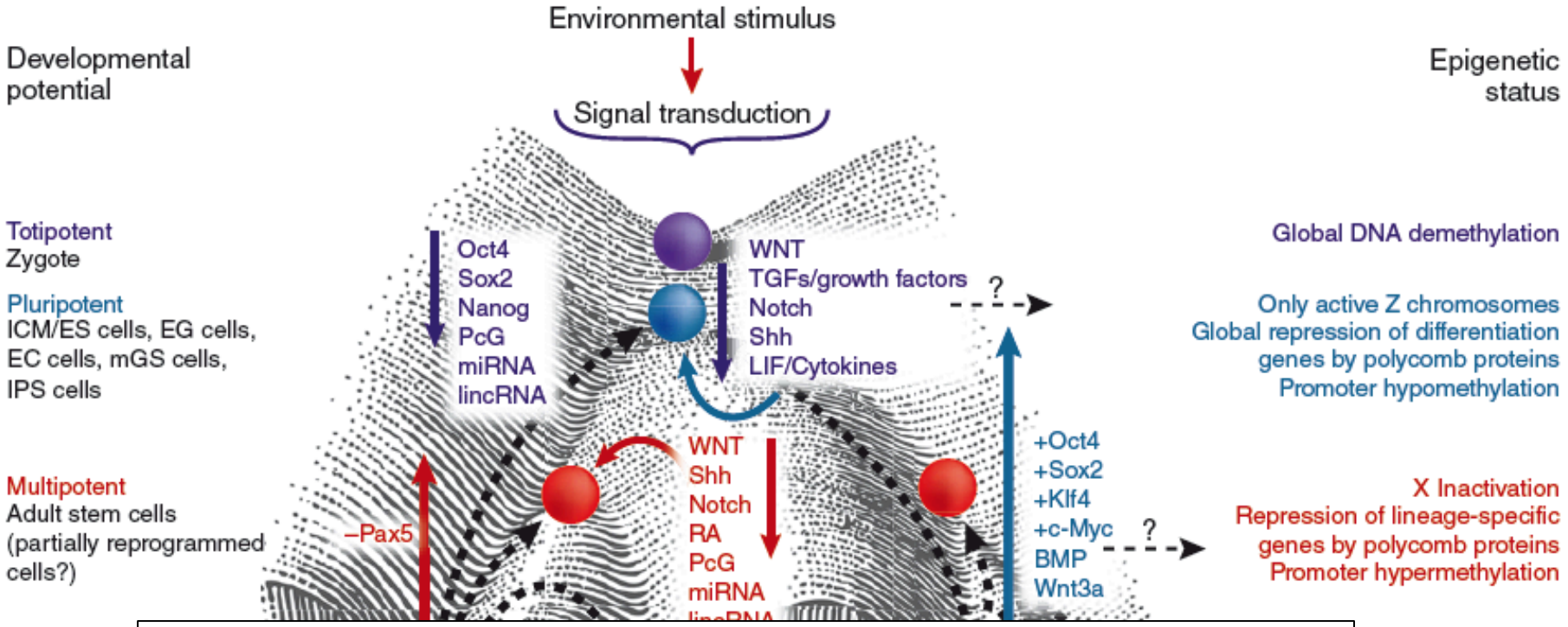


Buffering (canalization):
Up to a certain threshold, genetic or environmental variation will not affect the pathway

cf C.H. Waddington

Nevertheless, nutrition and other maternal influences clearly **can** have an impact on the developing fetus and its germ line…
(*more next week*)

Influence of envrionmental fluctuations during early mammalian development?



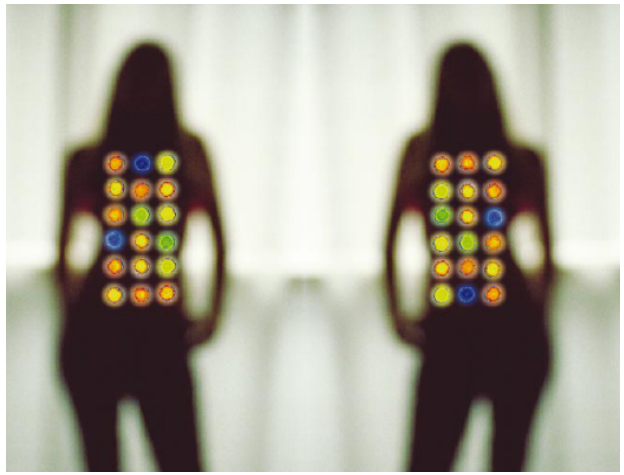
Epigenetic transitions play crucial roles in development and in the differentiation of stem cells and primordial germ cells.

Aberrant gain/loss of methylation may occur in early embryonic cells owing to external triggers or availability of the universal methyl donor, *S*-adenosylmethionine (SAM) which can affect *de novo* DNA methylation?

Epigenetic differences in Monozygotic Twins

Genetically identical…

… increasingly phenotypically
& epigenetically different with age?



Martin (2005) "Epigenetic drift in aging identical twin" *PNAS*, 102, 10413-10414

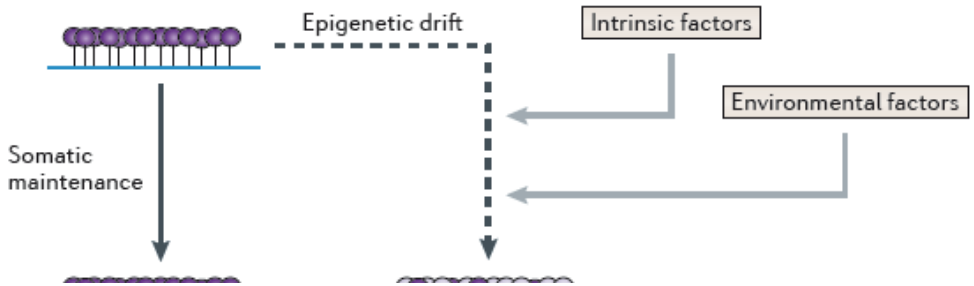
Aging and Epigenetic Drift

Increased changes in DNA Methylation with age over long time scales (Alisch et al, 2012; Christensen et a., 2009; Bollati et al, 2009; Boks et al., 2009; Rakyan et al., 2010; Bocklandt et al., 2011; Bell et al, 2012)

Changes in methylation have been linked to complex age-associated diseases such as metabolic disease (Barres and Zierath, 2011) and cancer (Jones and Laird, 1999; Esteller, 2008).

Studies have also observed "epigenetic drift," whereby the DNA methylation marks in identical twins increasingly differ as a function of age (Fraga et al., 2005; Boks et al., 2009).

- Due to *environmental* exposures that lead to predictable changes in epigenome?
- Due to *spontaenous* changes – through errors in copying DNA methylation or disruption of methylation



⇒ **One can measure human aging from DNA Methylomes**
Hannum et al (2013) Mol. Cell 49, 359–367.

Gender differences
Correlate with gene expression differences
Tumors show faster aging

CONCLUSIONS
Environmentally Induced Epigenomic Changes

Correlations versus causality?

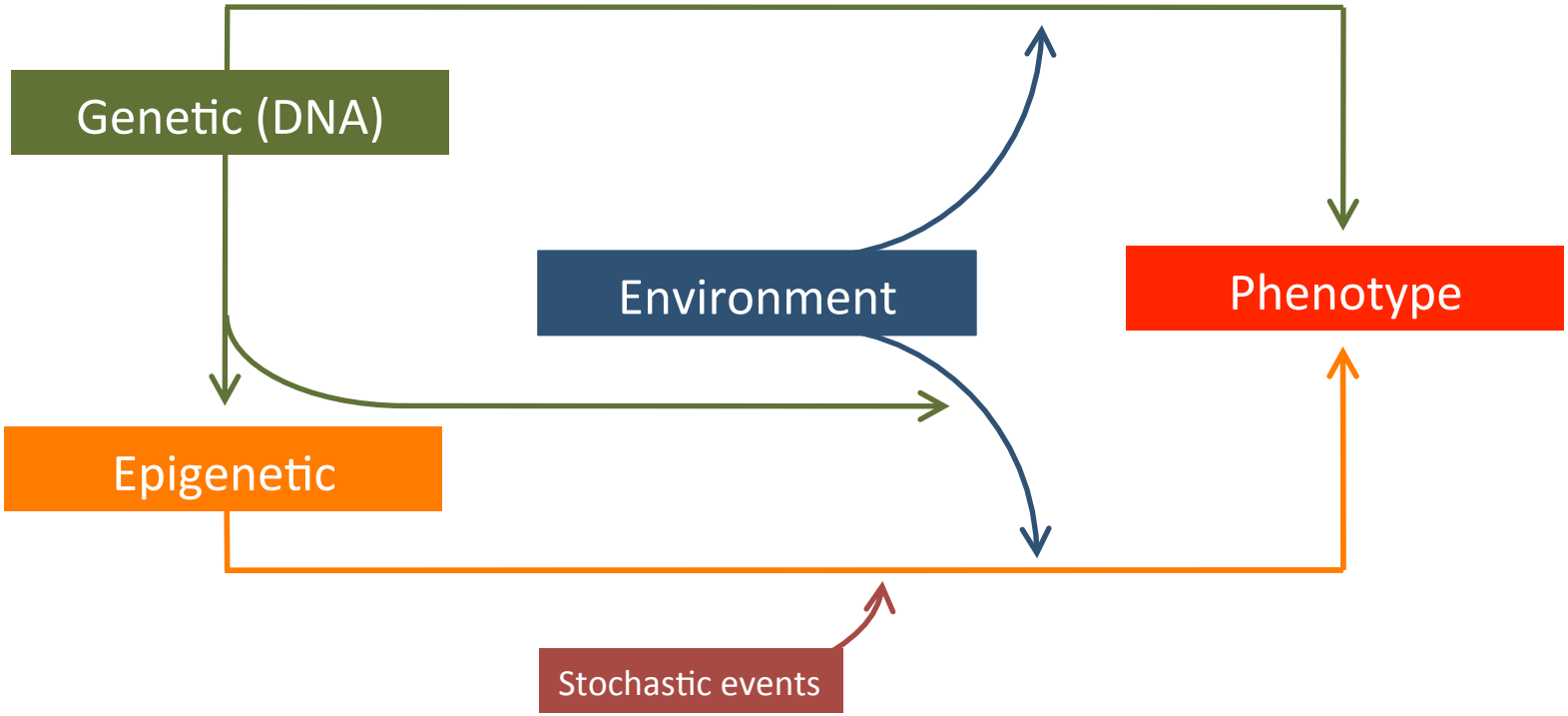
1. "Epigenetic" changes are often confounded with gene expression changes – epigenetics is not just about gene regulation…

2. So far, most "epigenetic" (epigenomic) changes can be used as biomarkers for specific states – developmental, age-related, behavioral, pathological etc - and ONLY if the correlations stand up to rigorous statistical testing)

3. How many of these changes are cause or consequence, or are linked to the state are unclear

4. Where epigenomic changes such as DNA methylation are established and subsequently maintained is still not clear - it is these processes on which environmental signals could have a considerable impact

How to define the nature and extent of the epigenetic components in environmentally-induced phenotypic changes?



Laboratory models:

- Genetically identical => *uniform* genetic information
- Can identify specific effects of different environmental influences
- Can identify the precise tume at which sensitutvity to the environment may occur
- Can identify the extent to which stochastic events contribute to phenotypic change