Research Article

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Epigenomics

Pilot study: limited evidence that common levels of prenatal alcohol exposure change the biology of the placenta; more evidence in male offspring but we need to look at many more samples (but need $$)

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Excellence in clinical care, research and education

The Royal Children's Hospital Melbourne

Murdoch Childrens Research Institute

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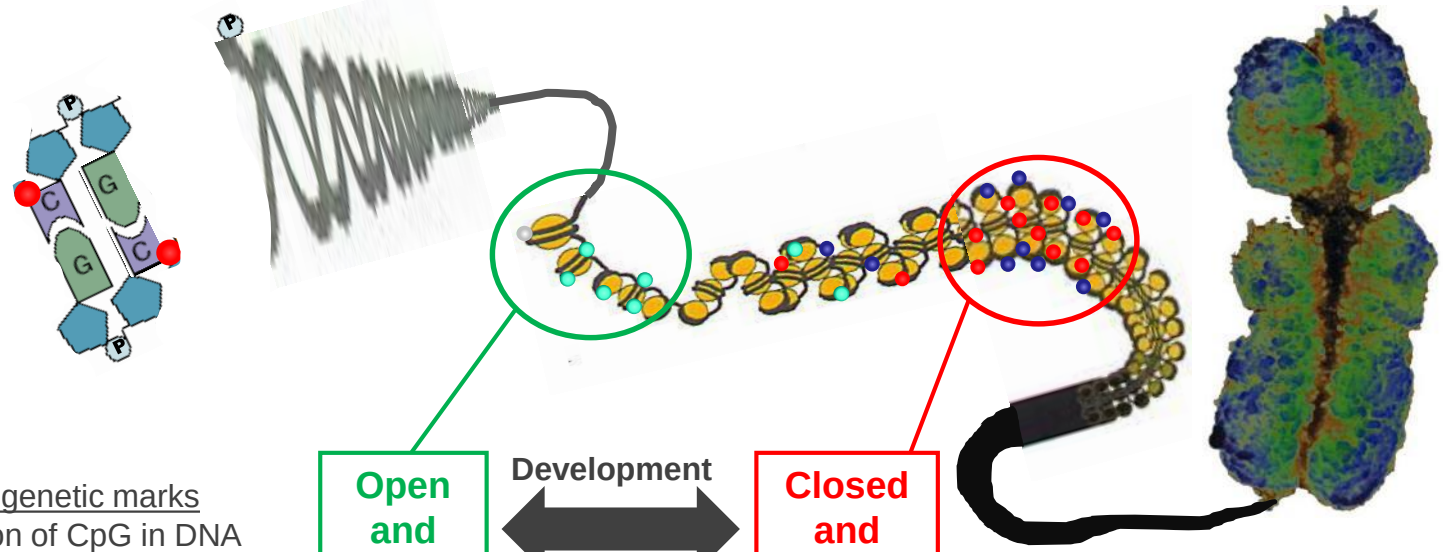
Epigenetic marks combine to open and close gene regulatory regions (on-off switches)

1. DNA

2. DNA with proteins (yellow)

3. Two metres of DNA per cell packaged with more proteins into chromosomes



Key to epigenetic marks
- Methylation of CpG in DNA
- Activating epigenetic molecules
- Inactivating epigenetic molecules

Open and active

Development

Environment

Closed and inactive

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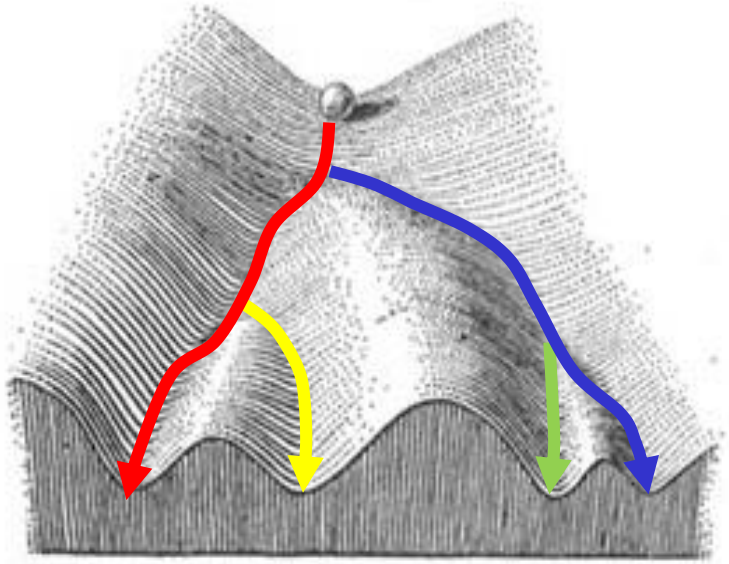
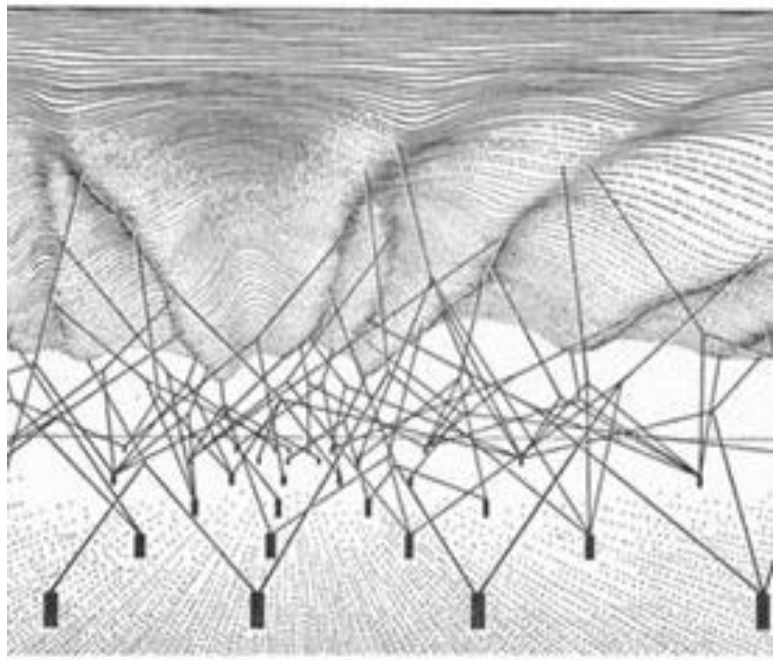
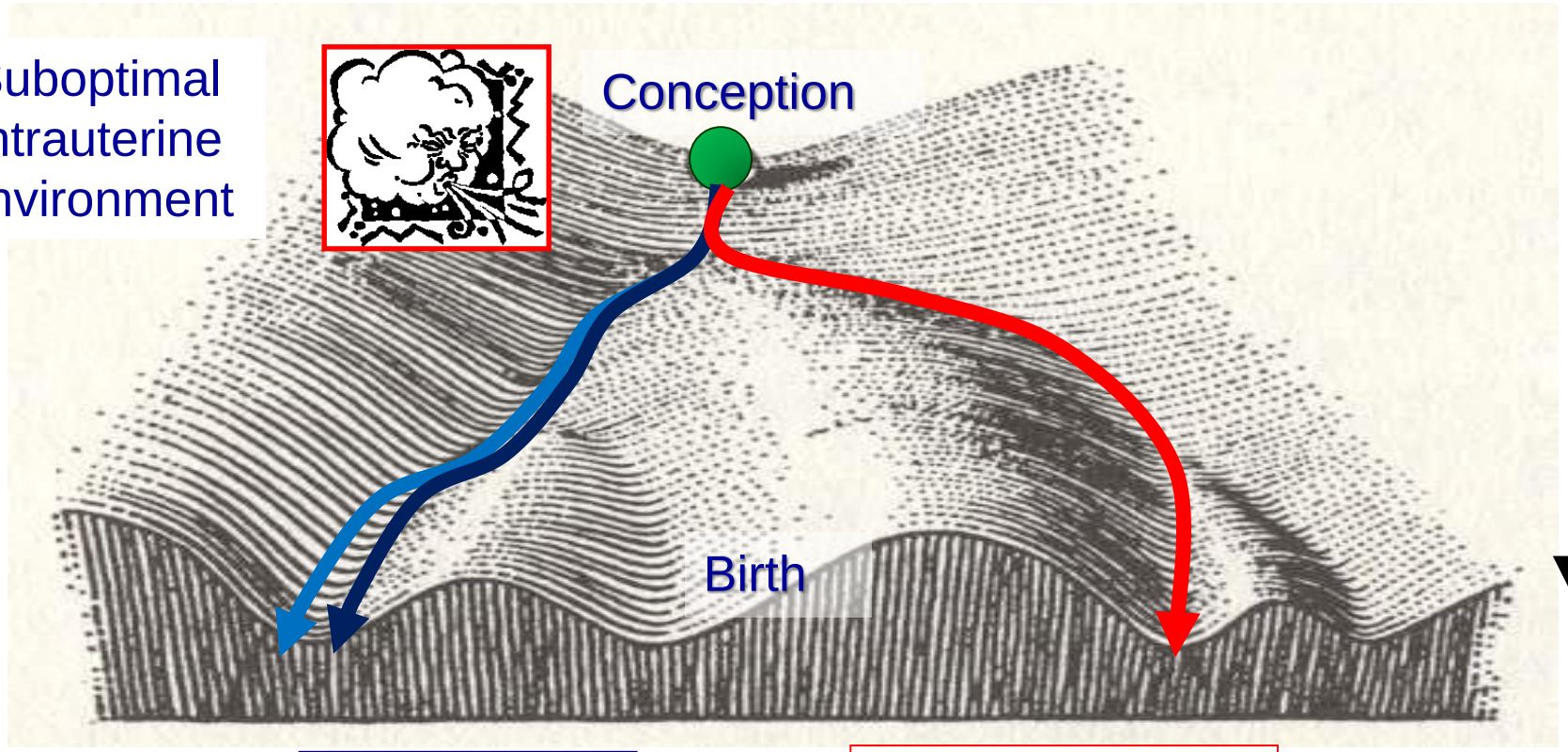
Epigenetics, development & Waddington's epigenetic landscape



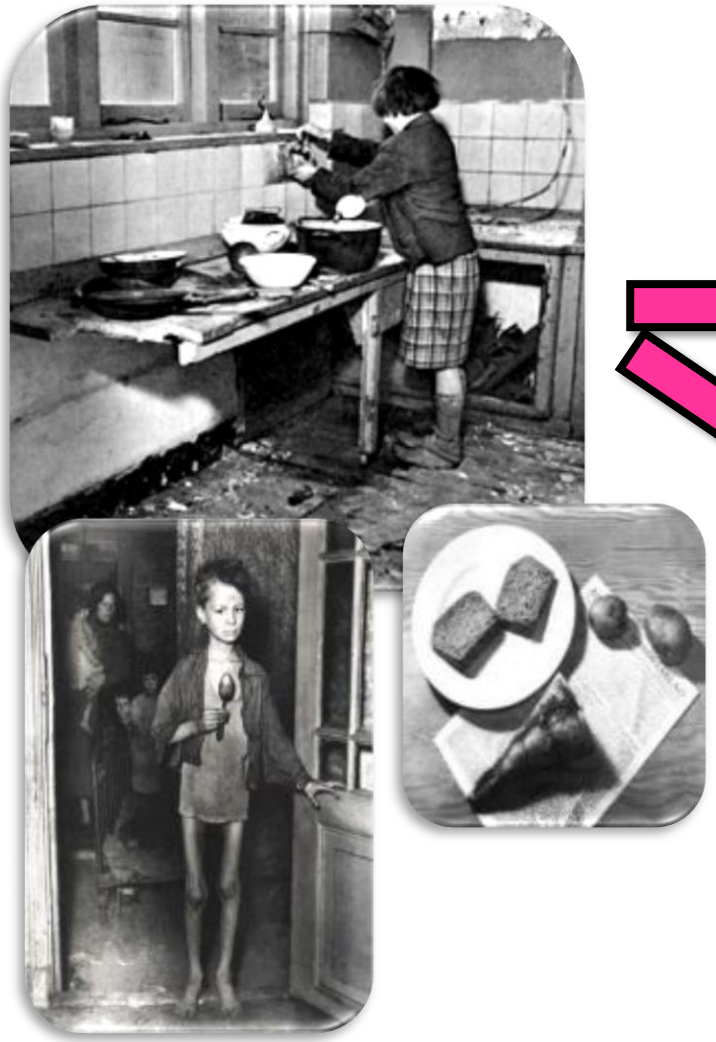
Development

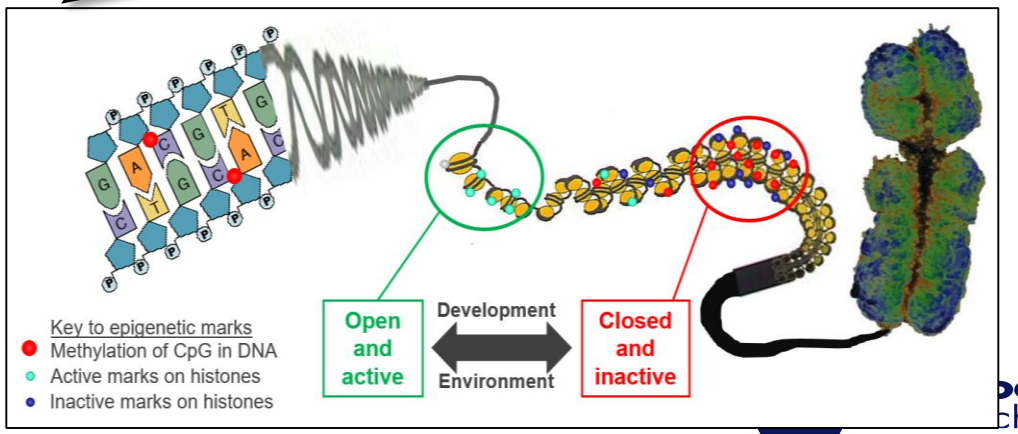


Waddington's 'Epigenetic Landscape'

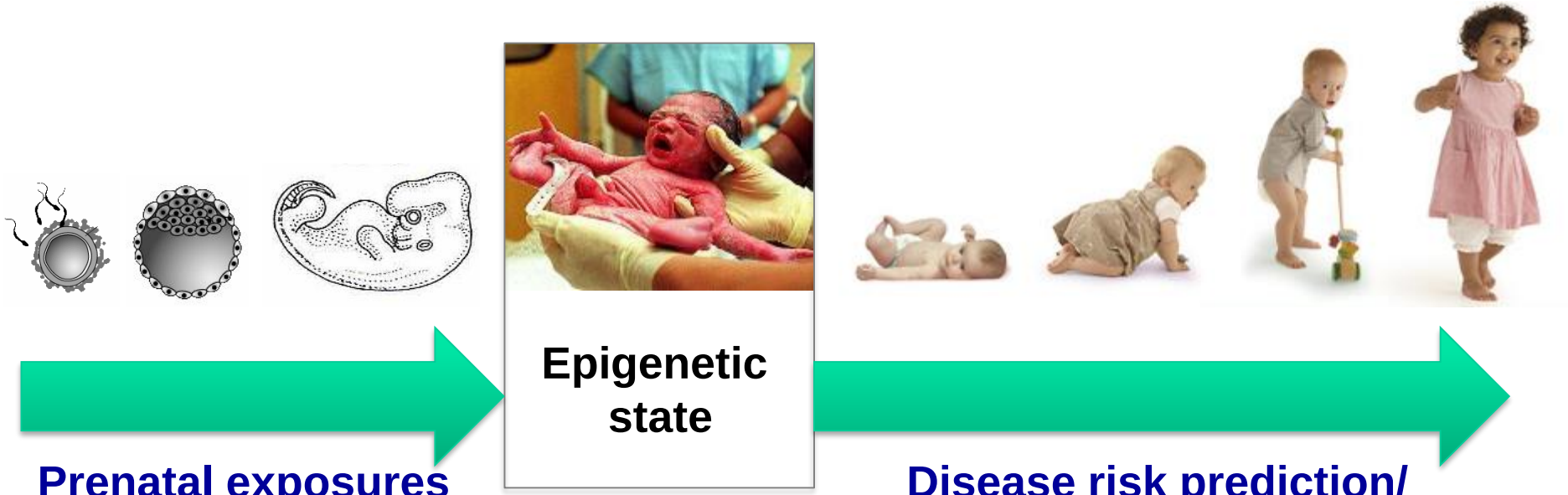


Epigenetics: mediator of early life environment on risk for chronic disease





Epigenetics: mediator of early life environment on risk for chronic disease



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Rationale

- Epigenetic change as a mediator of prenatal alcohol exposure (PAE) on adverse childhood outcomes
- Taking the first step
  - Previous studies of chronic alcohol exposure inc. FASD
  - Very few studies of dosage & timing of PAE

Hypothesis and Aim

- **Hypothesis**: epigenetic changes, in particular in the placenta, may mediate the effects of PAE on children's health.
- **Aim**: To examine the relationship between PAE patterns, based on dose & timing, and placental global DNA methylation.

- Alu interspersed repeat (canary in a coalmine)
- 11% genome
- Cancer
- Prenatal & postnatal toxicants

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AQUA

Asking questions about alcohol in pregnancy to determine infant health outcomes

Objectives

- Examine the effects of **dose** and **timing** of alcohol in pregnancy on specific physical and neurobehavioural outcomes in infants and young children

- Take into account important contextual factors that may help understand the heterogeneous nature of these effects; with special investigations into epigenetic and genetic influences

Measuring global DNA methylation

- Placenta, n=187
- ≥3 tech reps

Defined common patterns of alcohol exposure in 3 tiers

Tier 1	Abstainers	Any alcohol in pregnancy

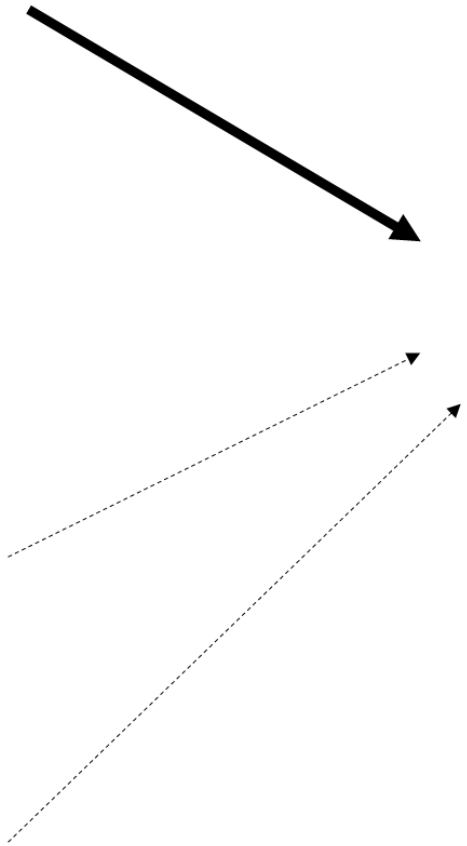
Statistical model

Prenatal Alcohol Exposure (PAE) groups

Maternal covariates:
Pre-pregnancy BMI
Age
Education
Household income per year
Presence of gestational diabetes
Smoking
Ethnicity
Healthy eating pattern scores
Folate intake
Partner's alcohol consumption
Mental and coping scores (AQoL)
Weight gain during pregnancy*

Infant covariates:
Sex
Gestational age
Birth weight

Batch effect*
(two SpectroCHIPs were used)



Global (Alu) DNA methylation

*associated with global DNA methylation

Univariate model

Tier 1	31 ab-stainers	Any alcohol in pregnancy; n=156; (1.3%, p=0.004)

Univariate model

Tier 1	**31 ab-stainers**	**Any alcohol in pregnancy; n=156; (1.3%, p=0.004)**	
Tier2	31 ab-stainers	Any level of alcohol in T1 only; n=77; (1.3%, p=0.009)	Any level of alcohol throughout pregnancy; n=79; (1.3%, 0.007)

Univariate model

Tier 1	**31 ab-stainers**	**Any alcohol in pregnancy; n=156; (1.3%, p=0.004)**					
Tier2	31 ab-stainers	Any level of alcohol in T1 only; n=77; (1.3%, p=0.009)			Any level of alcohol throughout pregnancy; n=79; (1.3%, 0.007)		
Tier 3	31 ab-stainers	Low; n=30; (0.6%, 0.3)	Mod.-high; n=25; (1.6%, 0.009)	Binge; n=22; (1.8%, 0.005)	Low in T1, low-mod. in T2/T3; n=15; (1.3%, 0.08)	Mod.-high in T1, low-high in T2/T3; n=28; (1.5%, 0.012)	Binge pre-aware, low-mod in T2/T3; n=36; (1.2%, 0.038)

Multivariate model

Adjusted for maternal weight gain during pregnancy, SpectroCHIP batch.

Tier 1	**31 ab-stainers**	**Any alcohol in pregnancy; n=156; (0.6%, p=0.108)**					
Tier2	31 ab-stainers	Any level of alcohol in T1 only; n=77; (0.6%, p=0.158)			Any level of alcohol throughout pregnancy; n=79; (0.7%, 0.117)		
Tier 3	31 ab-stainers	Low; n=30; (0.3%, 0.619)	Mod.-high; n=25; (0.7%, 0.19)	Binge; n=22; (1.0%, 0.074)	Low in T1, low-mod. in T2/T3; n=15; (0.8%, 0.211)	Mod.-high in T1, low-high in T2/T3; n=28; (0.90%, 0.084)	Binge pre-aware, low-mod in T2/T3; n=36; 0.50%, 0.34)

Multivariate model, males only

Adjusted for maternal weight gain during pregnancy, technical variable (batch effect).

Tier 1	**14 ab-stainers**	**Any alcohol in pregnancy; n=75; (1.0%, p=0.058)**	
Tier2	14 ab-stainers	Any level of alcohol in T1 only; n=33; (0.5%, p=0.342)	Any level of alcohol throughout pregnancy; n=42; (1.5%, 0.01)

Conclusions

Pilot study: limited evidence that common levels of prenatal alcohol exposure change the biology of the placenta; more evidence in male offspring but we need to look at many more samples (but need $$)

And need to consider <u>all</u> possible confounders, modifiers etc

Discussion

- Similar null findings to previous study
  - *Wilhelm-Benartzi et al Environ. Health Perspect. 120(2), 296–302 (2012).*
- Similar effect sizes to other environmental exposures
- Consistent with previous studies of sex-specific effects in placenta
- Functional consequences?

Future work

- Repeat in larger study, n>2000
- Epigenome-wide analysis

AQUA

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Early Life Epigenetics

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