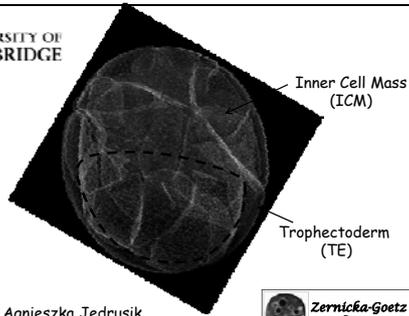


Role of maternal *Carm1* in early mouse embryo development

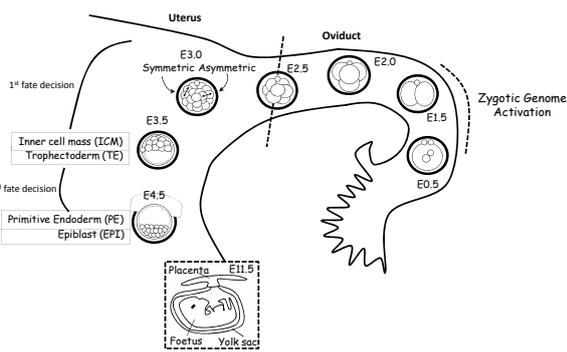
UNIVERSITY OF
CAMBRIDGE



Agnieszka Jedrusik
April 2012



Development of the early mouse embryo



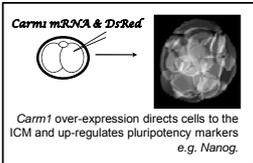
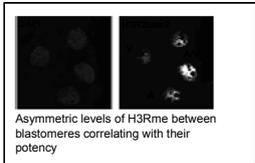
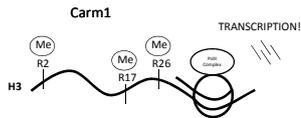
Maternal and zygotic 'control' of development

- Prior to zygotic genome activation (ZGA) embryonic development is dependent solely on maternally inherited RNAs and proteins (Braude et al. 1979; Bachvarova and De Leon 1980; Van Blerkom 1981; Cascio and Wassarman 1982; Bachvarova et al. 1985).
- In mouse, maternal factors control at least the first cell cycle, which is about 18-20 hours long (Howlett and Bolton 1985; Ciernych and Sicinski 2005).
- Maternal to zygotic transition (MZT) coincides with degradation of many but not all maternal transcripts.
- Up to 10% of labeled maternal mRNAs persist until the blastocyst stage, and as such can have functions at later developmental stages (Bachvarova and De Leon 1980).
- Oocyte-specific gene products, such as MATER (Tong et al. 2000) or FLOPED (Li et al. 2008), are expressed throughout pre-implantation stages.
- Maternally-derived proteins can rescue or delay phenotype expression in zygotic knockout model.

Carm1 (Prmt4)

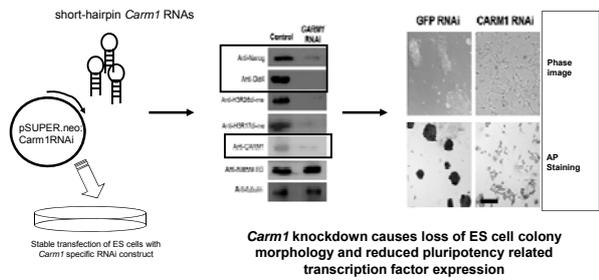
- Carm1 (Coactivator-associated arginine methyltransferase I; Prmt4), member of protein arginine methyltransferase family.
- Regulates gene expression by multiple mechanisms including methylation of histones and coactivation of steroid receptor transcription.
- In adults, Carm1 is overexpressed in human grade-III breast tumors and prostate adenocarcinomas, and knockdown of Carm1 inhibits proliferation of breast and prostate cancer cell lines.
- In early mouse development it is associated with cell pluripotency.

Carm1 (Prmt4) - arginine methyltransferase associated with pluripotent cell fate



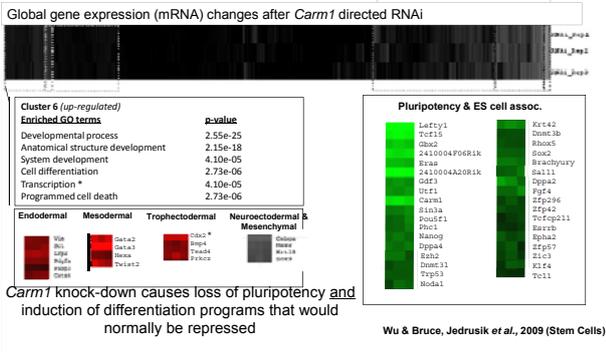
Torres-Padilla et al., 2007 (Nature)

Carm1 'loss of function' experiments in ES cells

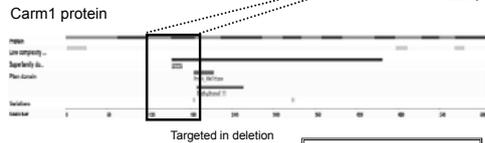
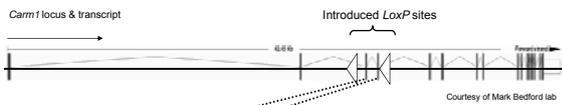


Wu & Bruce, Jedrusik et al., 2009 (Stem Cells)

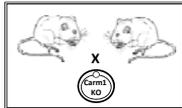
Microarray transcript profile suggests *Carm1* KD initiates ES cells differentiation



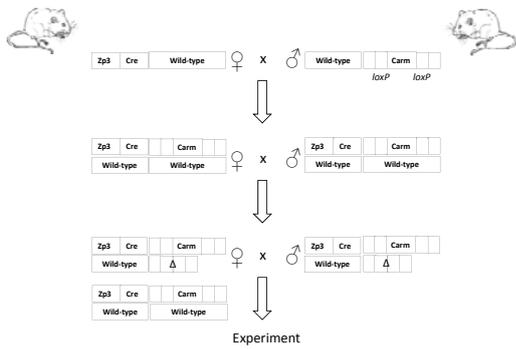
Targeting the *Carm1* locus in early embryo



Targeted deletion of the 3rd & 4th exons (amino acids 117-187) results in a non-functional, catalytically dead, *Carm1* protein with disrupted active site unable to bind the AdoMet cofactor.



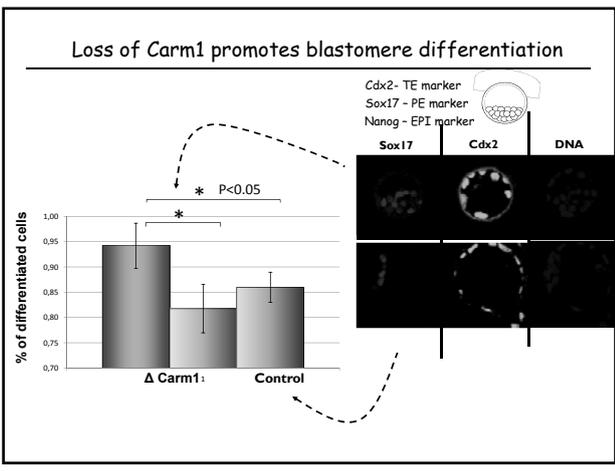
Carm1 KO embryos - experimental strategy

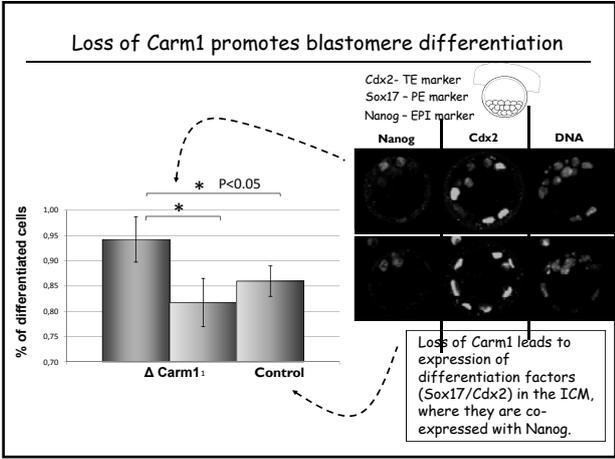


Loss of *Carm1* expression affects developmental progression

		Total	Blastocyst (cell death)	Arrested
<i>Δ Carm1</i>	1	41	18 (7)	23
	2	29	21 (5)	8
	3	28	24 (7)	4
<i>Carm1 control</i>	1	38	37 (4)	1
	2	NA	NA	NA
	3	46	43 (4)	3

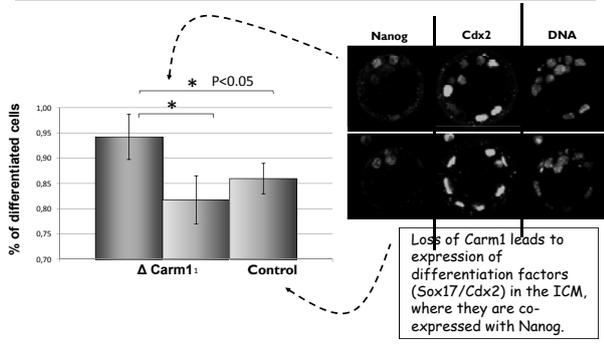
Cdx2- TE marker
 Sox17 - PE marker
 Nanog - EPI marker





Loss of maternal Carm1 promotes blastomere differentiation

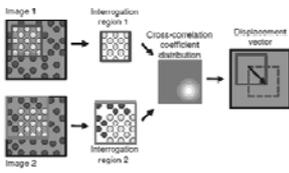
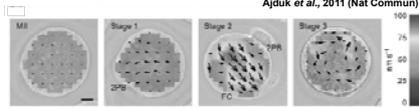
Differentiation 'defects' are observed regardless of paternal genotype.



Assessing embryo quality

Particle Image Velocimetry (PIV)

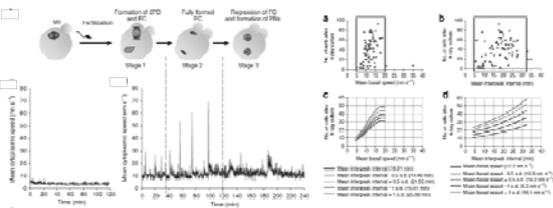
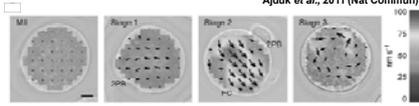
Ajduk et al., 2011 (Nat Commun)



Assessing embryo quality

Particle Image Velocimetry (PIV)

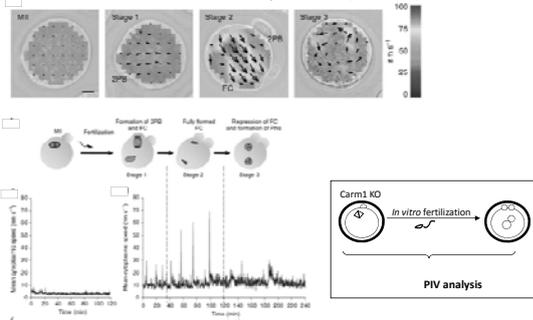
Ajduk et al., 2011 (Nat Commun)



Can we predict KO phenotype?

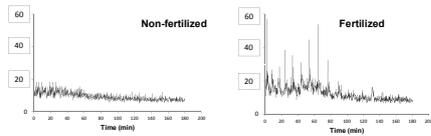
Particle Image Velocimetry (PIV)

Ajduk et al., 2011 (Nat Commun)



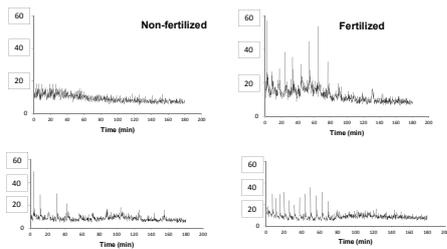
Can we predict KO phenotype?

Particle Image Velocimetry (PIV)



Can we predict KO phenotype?

Particle Image Velocimetry (PIV)



Conclusions:

- Maternal *Carm1* is critical for early mouse development.
- Loss of *Carm1* affects embryo and ES cells potency and leads to cell differentiation.
- Particle Image Velocimetry analysis provides promise for identifying mutant embryos.

Acknowledgements

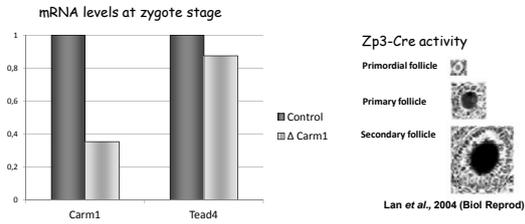


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Krzysztof Wicher
Anna Ajduk
MZG group
Martin Johnson
Gurdon Institute*





Efficiency of maternal Carm1 depletion



Loss of maternal Carm1 promotes blastomere differentiation

Differentiation 'defects' are observed regardless of paternal genotype.

