An Epigenetic Pathway Specifies Phenotype in Breast Cancer

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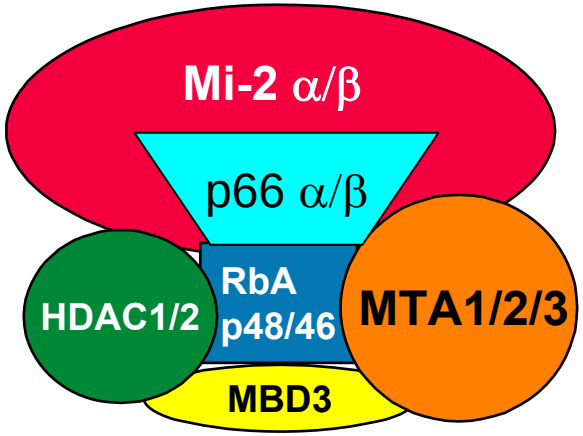
Research Focus – Mi-2/NuRD complex



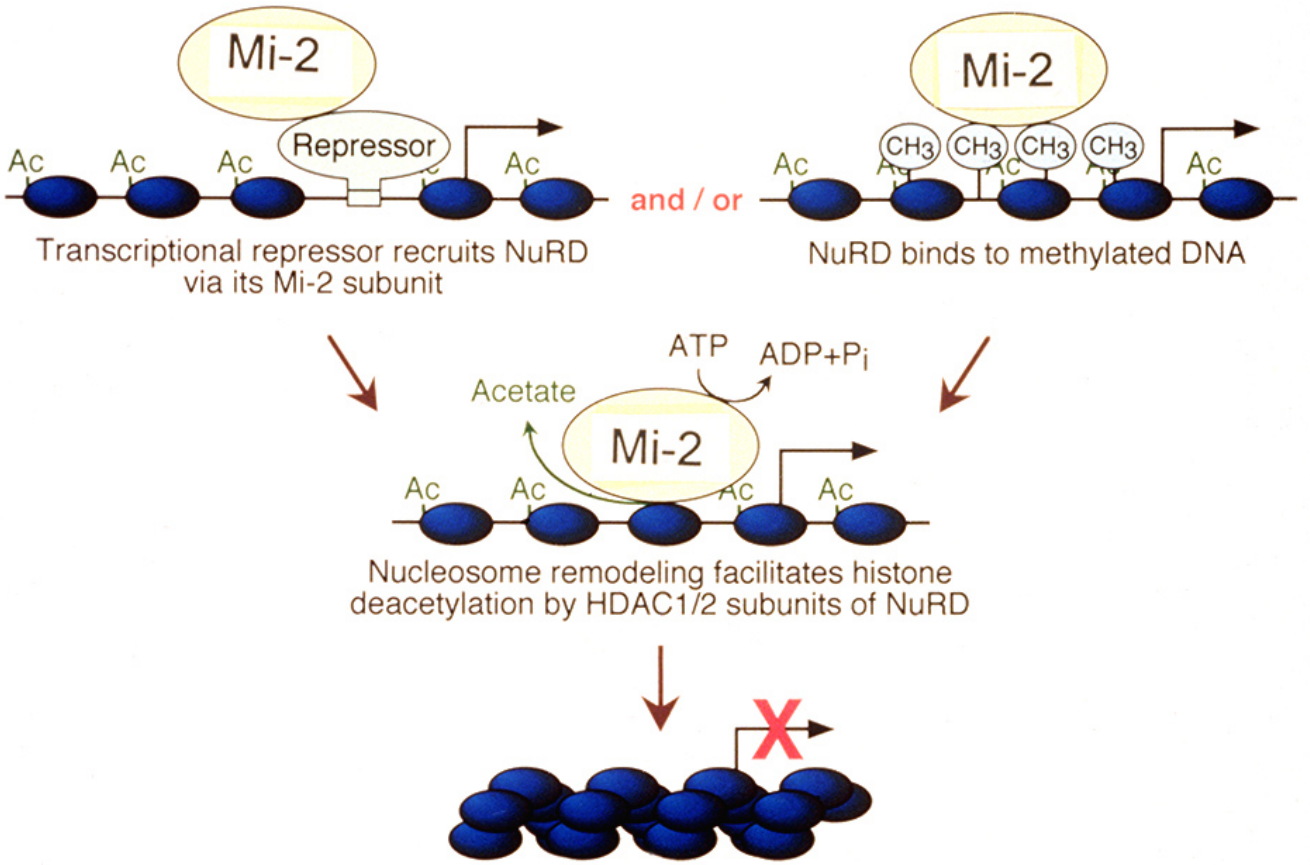
- **Chromatin remodeling complex**
- **Alters modification status of core histones through HDAC activity**

Laboratory Research Themes:

We study how gene regulatory events mediated by epigenetic mechanisms impact the following biological systems:

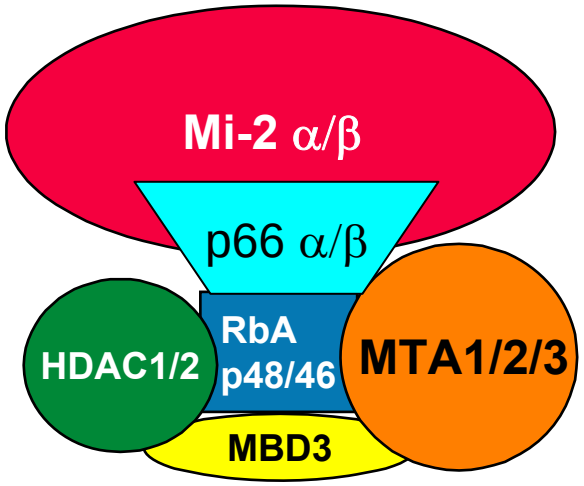
1. **Breast cancer**
2. **B lymphocyte development and disease**

The Mi-2/NURD Complex: Transcriptional Repression



Tyler and Kadonaga Cell 1999

The vertebrate MTA protein family



- Metastasis associated proteins
- Three genes in all vertebrate genomes
- Multiple protein products (most mRNAs alternatively spliced)
- Most protein products associated with NuRD


BAH Domain
MTA1S MAANMYRVGDYVYFENSSSNPYLIRRIEELNKTANGNVEAKVVCFYRRRDISSTLIALADKHATLSVCYKAGPGADNGEEGEIEEEMENPEMVDLPEKLK*********
MTA1 MAANMYRVGDYVYFENSSSNPYLIRRIEELNKTANGNVEAKVVCFYRRRDISSTLIALADKHATLSVCYKAGPGADNGEEGEIEEEMENPEMVDLPEKLK*********
MTA2 MAANMYRVGDYVYFENSSSNPYLVRRIEELNKTANGNVEAKVVCLFRRRDISSSLNSLAD**SNAREFEEE
MTA3 MAANMYRVGDYVYFENSSSNPYLIRRIEELNKTASGNVEAKVVCFCRRRDISNTLIMLADKHAK*****************EIEEESETTVEADLTDKQK*********
MTA3L MAANMYRVGDYVYFENSSSNPYLIRRIEELNKTASGNVEAKVVCFCRRRDISNTLIMLADKHAK*****************EIEEESETTVEADLTDKQK*********
BAH Domain
ELM Domain
MTA1S **********HQLRHRELFLSRQLESLPATHIRGKCSVTLLNETESLKSYLEREDFFFYSLVYDPQQKTLLADKGEIRVGNRYQADITDLLKEGEEDGRDQSRLETQV
MTA1 **********HQLRHRELFLSRQLESLPATHIRGKCSVTLLNETESLKSYLEREDFFFYSLVYDPQQKTLLADKGEIRVGNRYQADITDLLKEGEEDGRDQSRLETQV
MTA2 SKQPGMSEQQRHQLKHRELFLSRQFESLPATHIRGKCSVTLLNETDILSQYLEKEDCFFYSLVFDPVQKTLLADQGEIRVGCKYQAEIPDRLVEGESDNRNQQKMEMKV
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ELM Domain
SANT Domain
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SANT Domain
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Zinc Finger
MTA1S ILLDILEEIWWLENANPVRWREARTKPQ
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Zinc Finger
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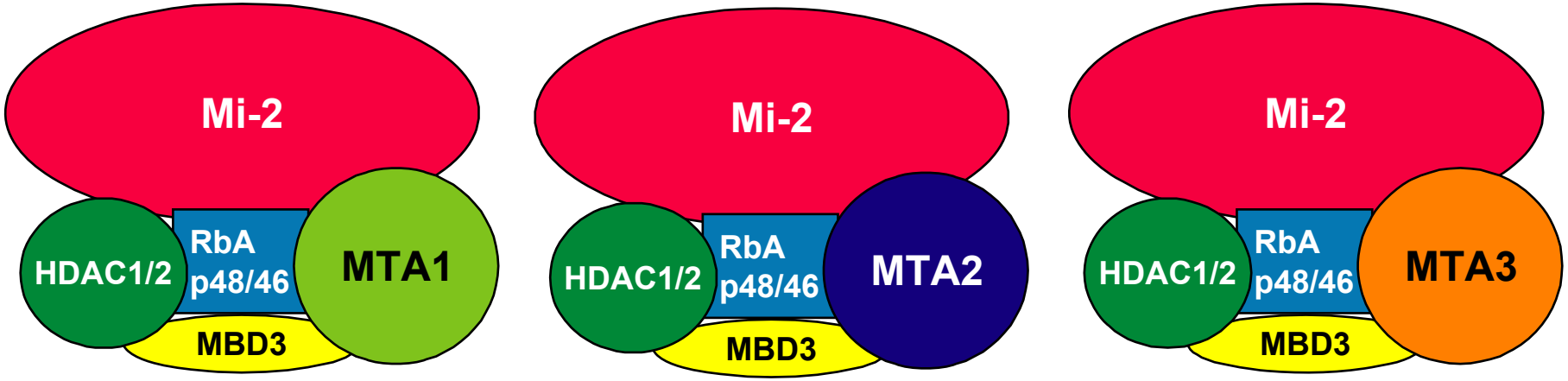
MTA3L PFVAINYAAIRAEYADRHAELSGSPLKSKSTRKPLACIIGYLEIHPAKKPNVIRSTPSLQTPTTKRMLTTPNHTSLSILGKRNYSHHNGLDELTCCVSD
MTA1 NHGQTRHMGPSRNLLLNGKSYPTKVRLIRGGSLPPVKRRRMNWIDAPGDVFYMPKEETRKIRKLLSSSETKRAARRPYKP*****IALRQSQALPPRPPPPAPVNDEPI
MTA2 **************************IRSSSQPAAKRQKLNPADAPNPVVFVATKDTRALRKALTHLEMRRAARRPNLPLKVKPTLIAVRPPVPLPAPSHPASTNEPI
MTA1 VIED
MTA2 VLED

Working hypothesis: MTA family members are alternative subunits of unique complexes



-This hypothesis predicts that unique complexes have specialized functions

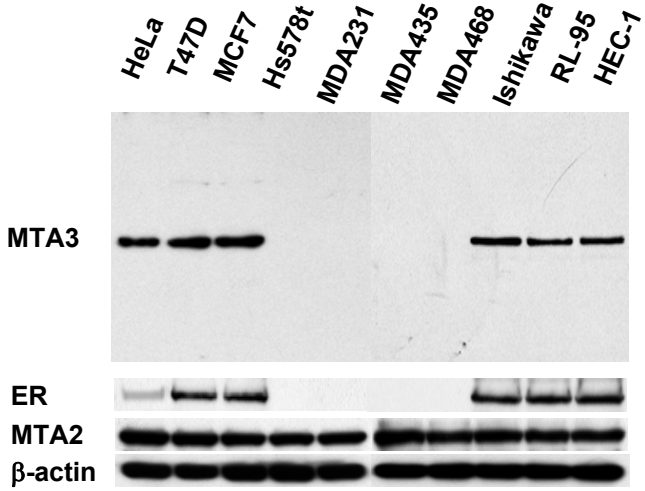
-HDAC presence implies gene regulation by modulation of histone acetylation

Mi-2/ NuRD complex in breast cancer

1. Subunit composition of the Mi-2/NuRD complex responds to cellular environment

2. This complex is a component of a genetic regulatory circuit that impacts cell shape, physiology, and behavior

MTA3 expression correlates with ER status in human cell lines



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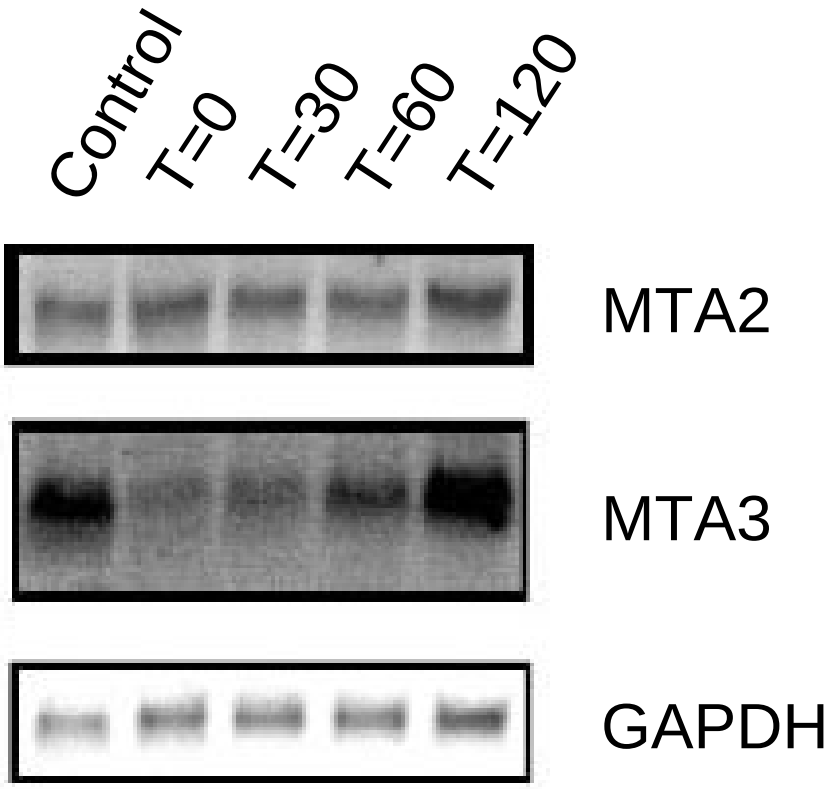
MTA3 mRNA levels are estrogen dependent



- Deprive cells of steroids by culturing in stripped media
- add back estradiol - harvest cells over time
- **RESULTS**
  - MTA3 mRNA levels decrease in the absence of steroids
  - levels are fully restored following addition of estradiol
  - kinetics of transcript accumulation suggest that MTA3 is not directly regulated by ER
  - MTA2 is insensitive to estradiol

Naoyuki Fujita

Working Model

ER-α ON

↓

MTA3 ON

⊣

Transcriptional targets
Off

↓

Functions???

ER-α OFF

✗↓

MTA3 OFF

✗⊣

Transcriptional targets
On

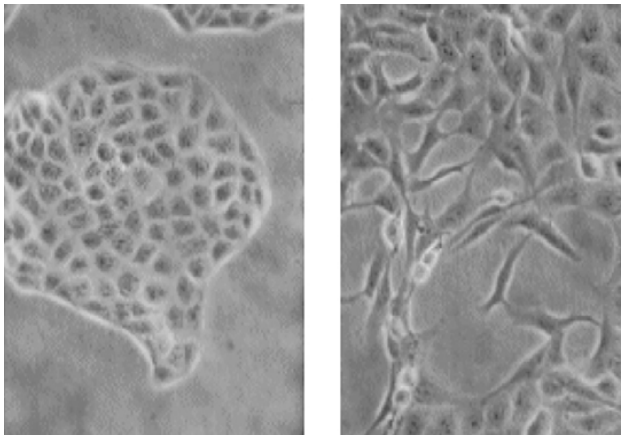
↓

Functions???

Fujita et al., 2003, Cell 113, 207-219
Fujita et al., 2004, Mol Endo 18, 2937-2949

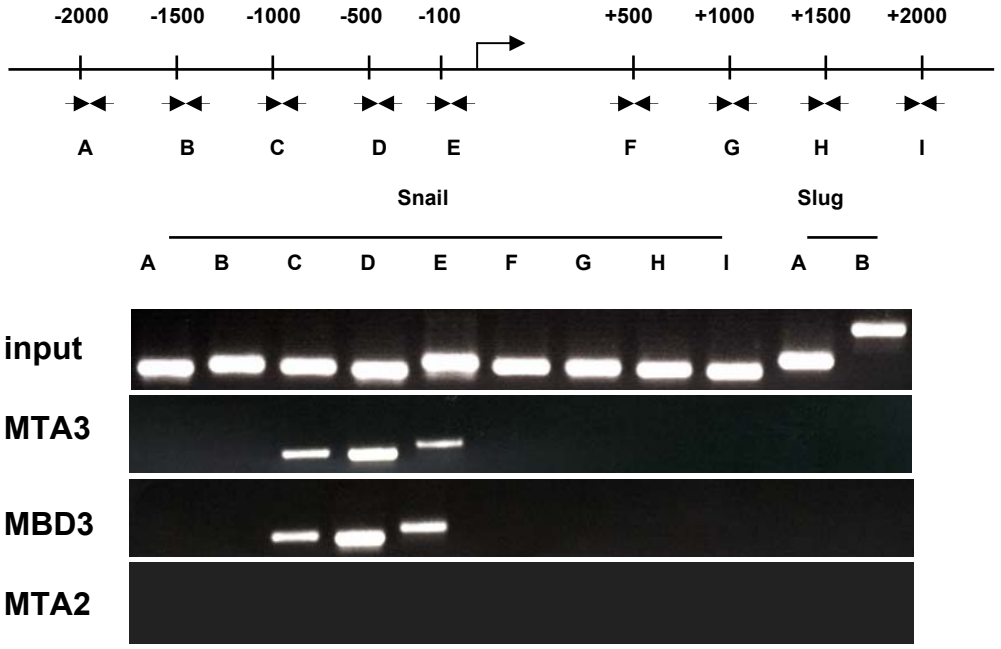
Snail – a candidate Mta3 target

- In Drosophila, snail is involved in establishment of the dorsal-ventral axis
- In snail mutants, epithelial cells fail to change cell fate and migrate at appropriate developmental time
- In mammals, snail is a direct transcriptional repressor of E-cadherin – a marker for epithelial cells



J Biol Chem. 2002 Oct 18;277(42):39209

MTA3 localizes just upstream of the transcription start site at Snail



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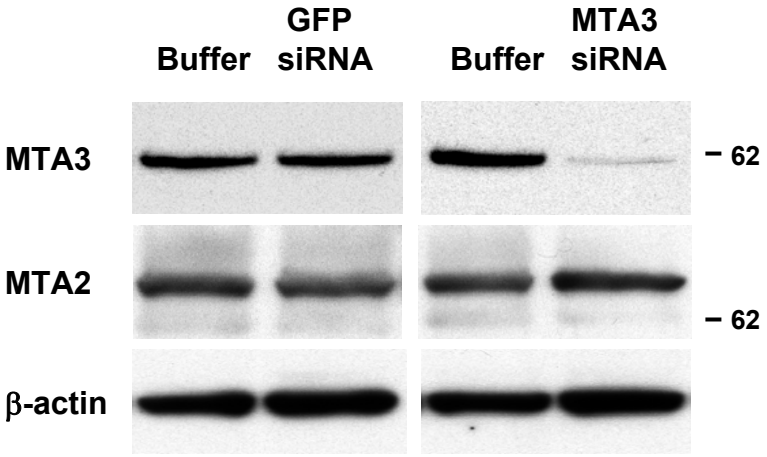
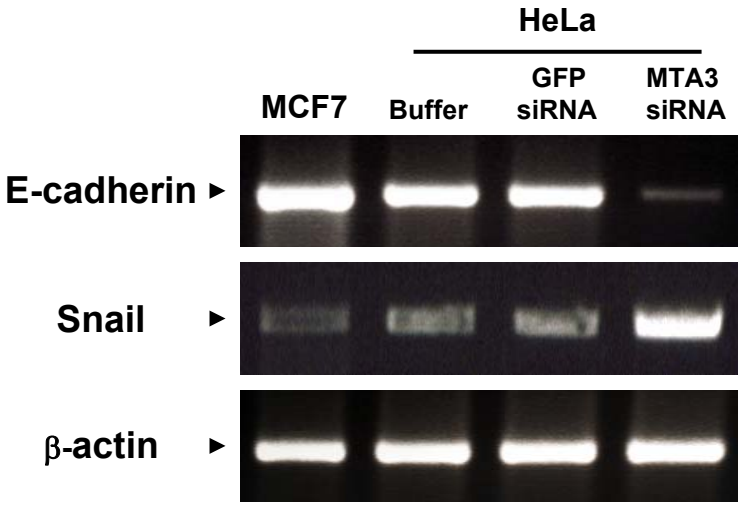
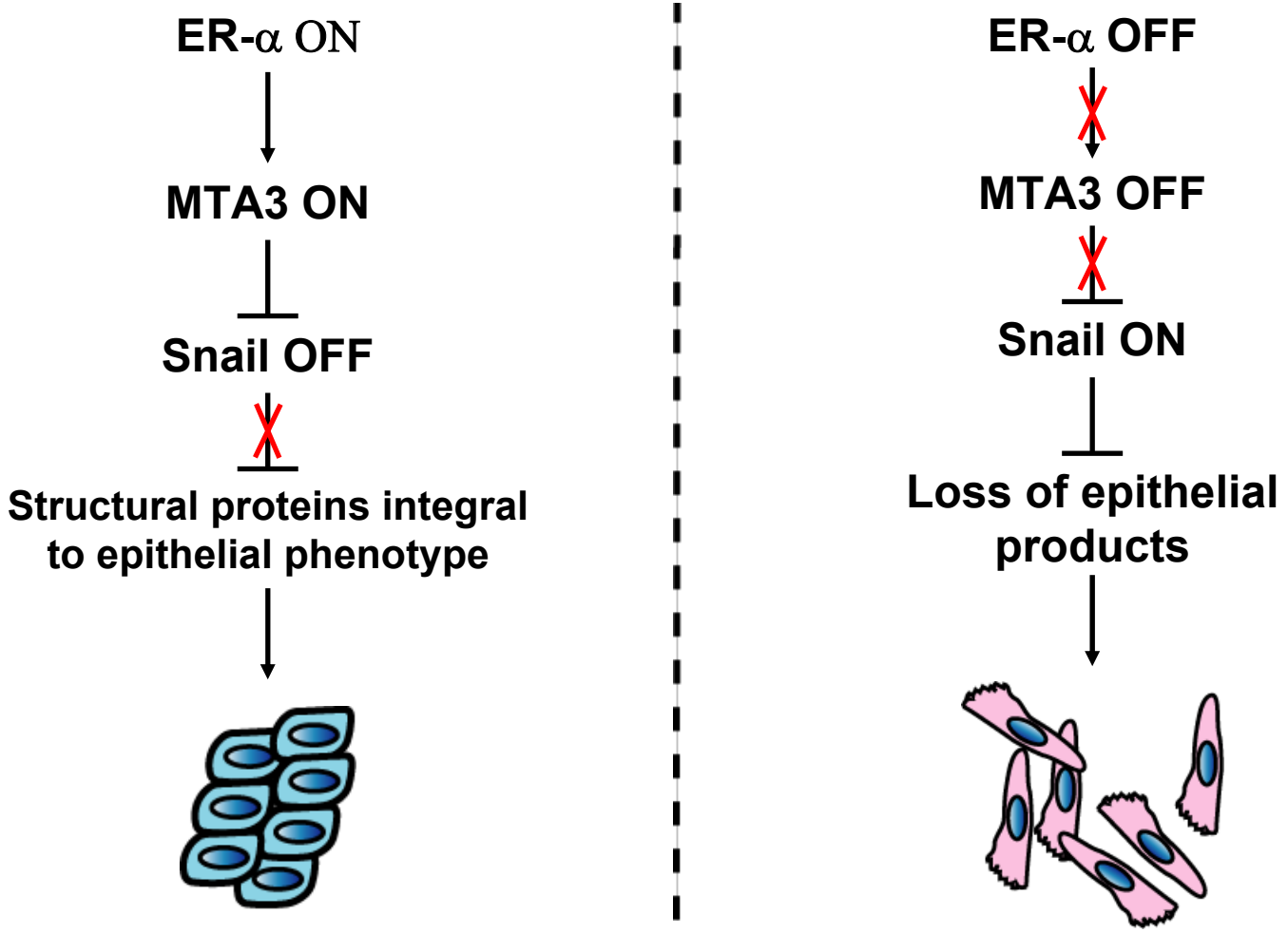
Depletion of MTA3 by RNAi leads to increased transcription of Snail





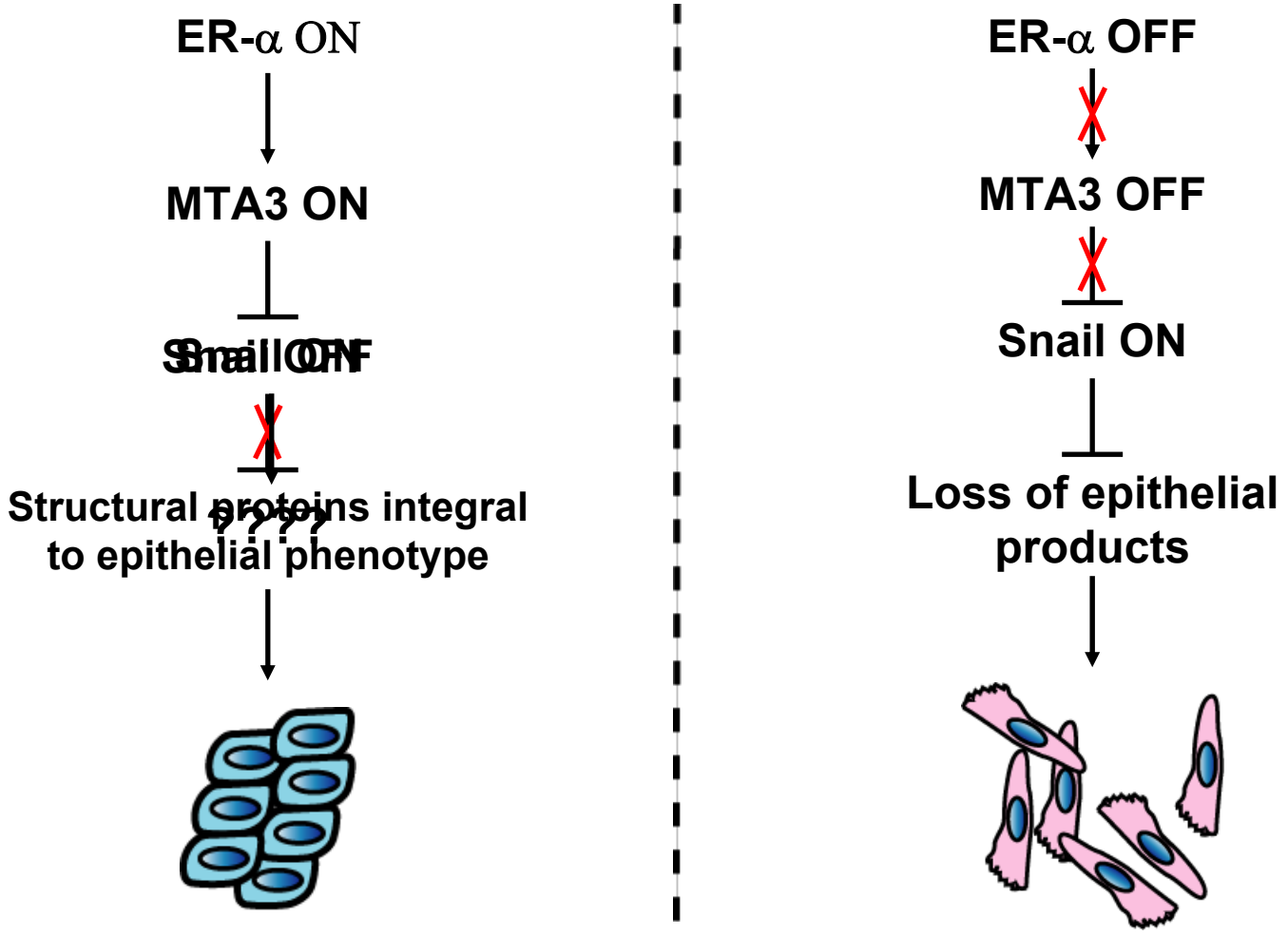
Naoyuki Fujita

The estrogen receptor, MTA3, Snail genetic pathway in breast cancer



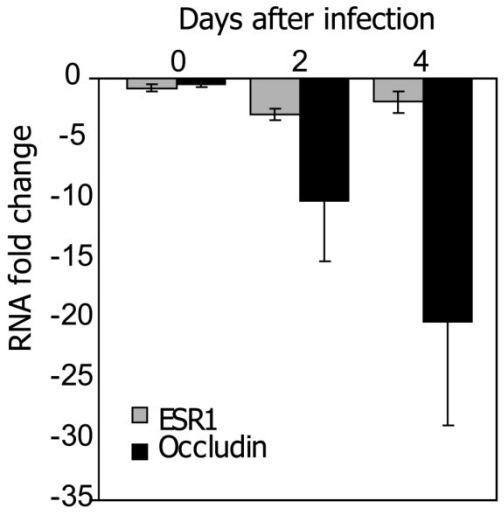
Fujita et al., 2003, Cell 113, 207-219
Fujita et al., 2004, Mol Endo 18, 2937-2949

What are the consequences of aberrant Snail expression in ER-α positive cells?

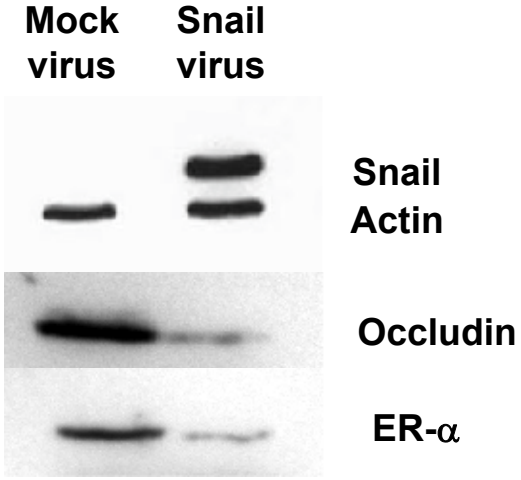


Aberrant Snail expression leads to loss of expression of epithelial products and ER-α

RT-PCR

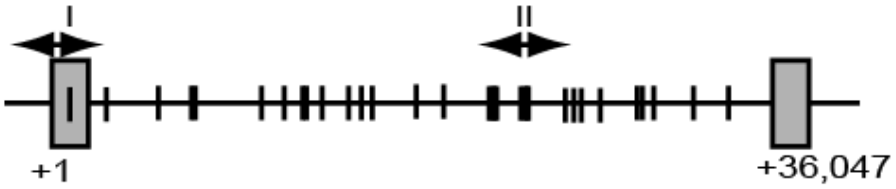


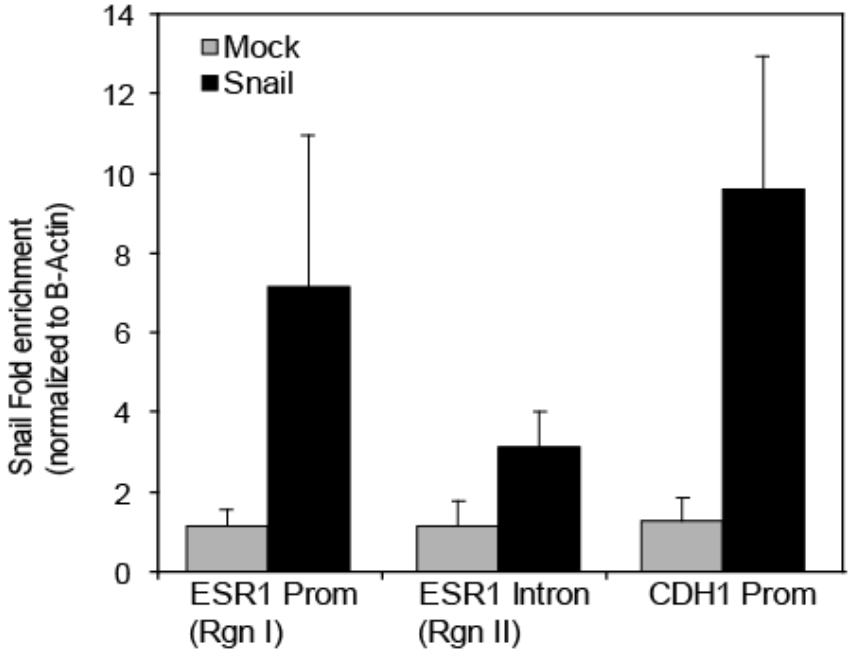
Immunoblot



Archana Dhasarathy

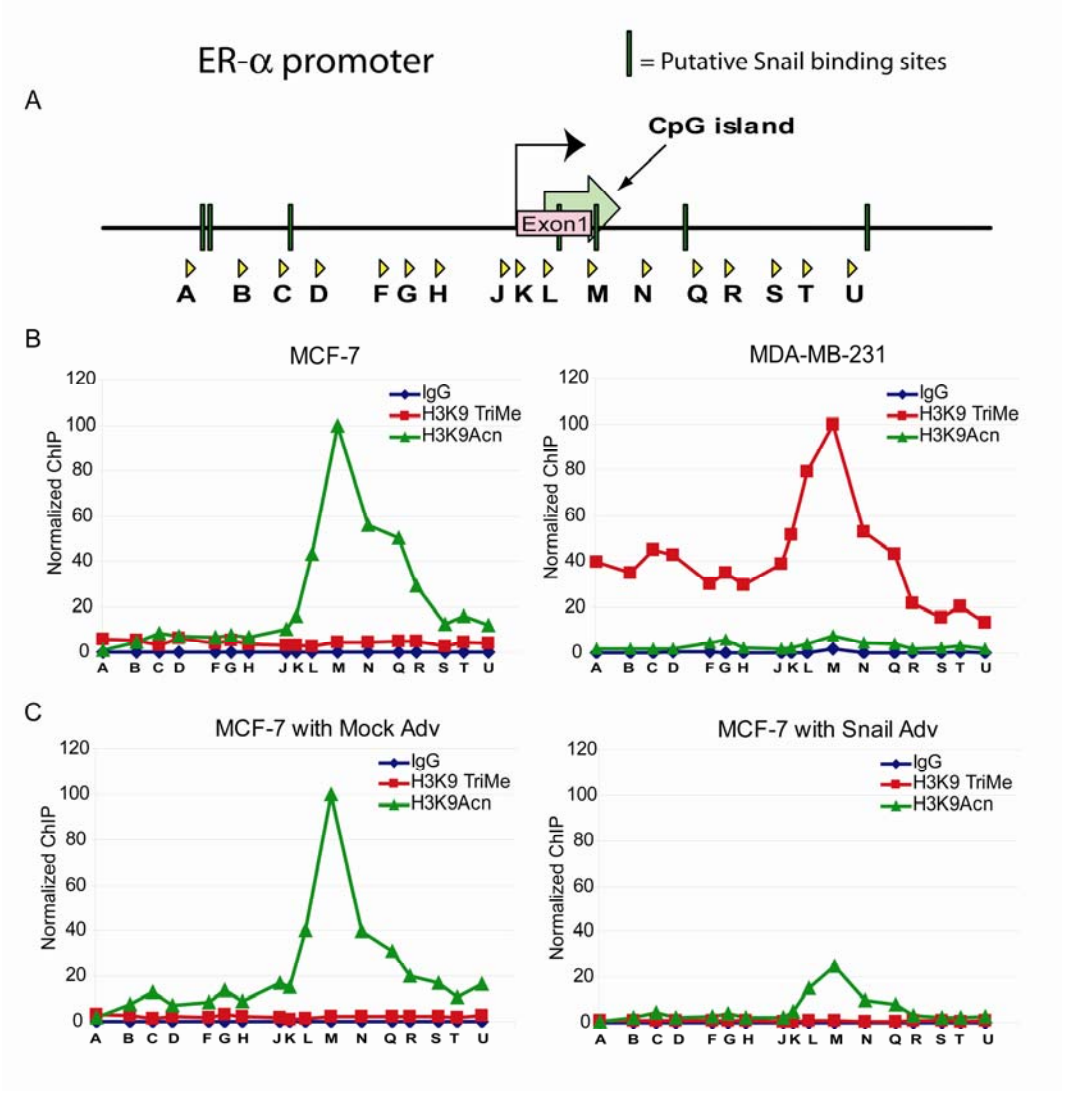
Snail directly binds the *ESR1* locus





Archana Dhasarathy

Snail binding induces alterations in local chromatin features at ESR1



Archana Dhasarathy

A complex genetic circuit regulating cell physiology at the level of chromatin

ER-α ON

↓

MTA3 ON

⊣

Snail OFF

✕

Structural proteins integral to epithelial phenotype

↓

ER-α OFF

✕

MTA3 OFF

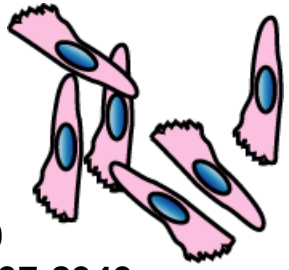
✕

Snail ON

⊣

Loss of epithelial products

↓



Fujita et al., 2003, Cell 113, 207-219
Fujita et al., 2004, Mol Endo 18, 2937-2949
Dhasarathy et al., 2007, Mol Endo 21, 2907-2918

Important aspects of this model are consistent with gene expression data from human tumors

- **Analysis of microarray data sets indicates a negative correlation between Snail and ER-α**
  - Wang et al -0.25 (p=1.6 E-04)
  - Sotiriou et al -0.44 (p=7.2 E-10)
  - Hess et al -0.40 (p=1.15 E-05)
- **Suggests that important regulatory features predicted in studies of cultured cell lines are upheld in tumors**

Do other environmental cues impact Mi-2/NuRD?

Mi-2/ NuRD complex in breast cancer

1. Subunit composition of the Mi-2/NuRD complex responds to cellular environment

2. This complex is a component of a genetic regulatory circuit that impacts cell shape, physiology, and behavior

NIEHS - current

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- Chris Malone
- Jennifer Sims

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