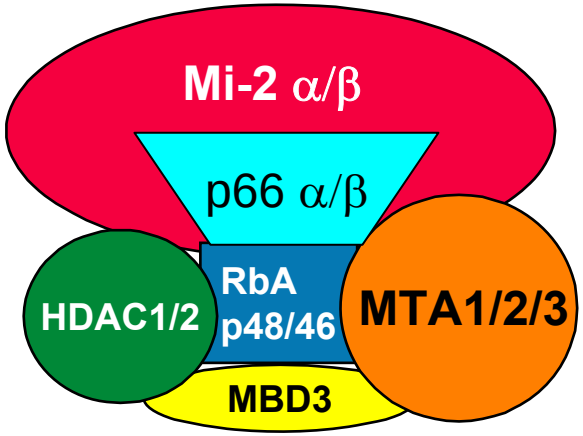
An Epigenetic Pathway Specifies Phenotype in Breast Cancer

Paul Wade
Laboratory of Molecular Carcinogenesis

Research Focus – Mi-2/NuRD complex



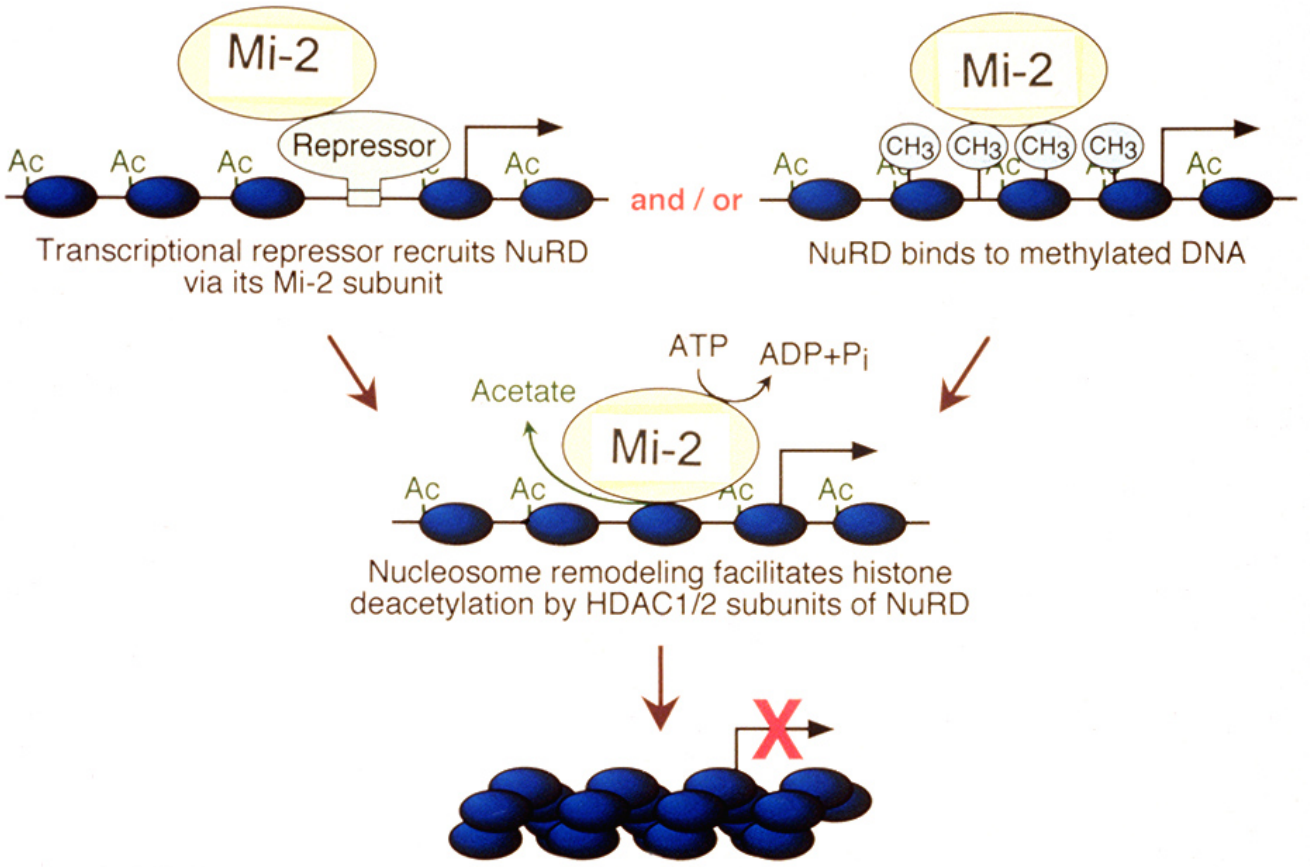
- **Chromatin remodeling complex**
- **Alters modification status of core histones through HDAC activity**

Laboratory Research Themes:

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

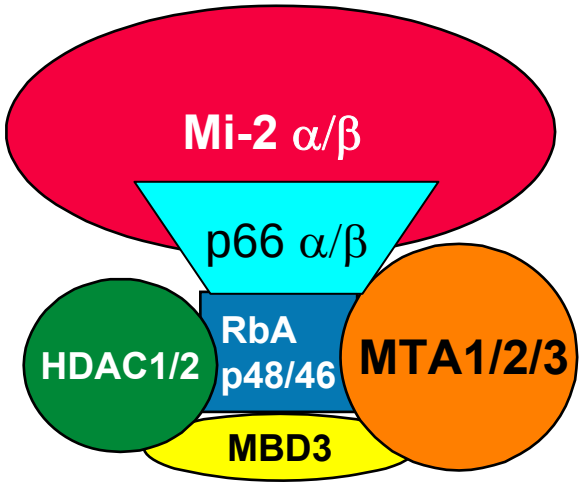
1. **Breast cancer**
2. **B lymphocyte development and disease**

The Mi-2/NURD Complex: Transcriptional Repression



Tyler and Kadonaga Cell 1999

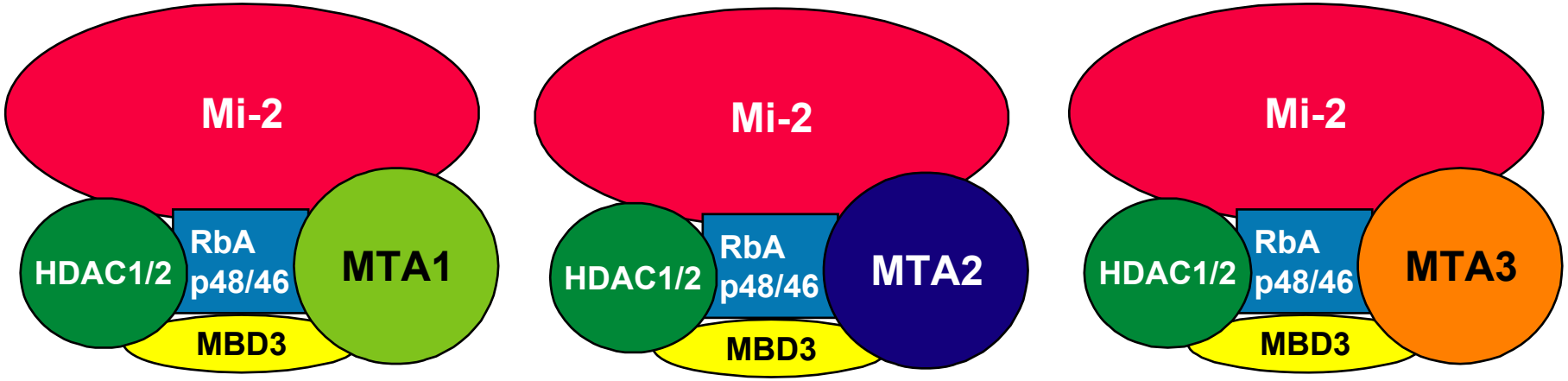
The vertebrate MTA protein family



- 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
MTA3 **********HQLKHRDLFLSRQYESLPATHIRGKCSVALLNETESVLSYLDKEDTFFYSLVYDPSLKTLLADKGEIRVGPRYQADIPEMLLEGESDEREQSKLEVKV
MTA3L **********HQLKHRDLFLSRQYESLPATHIRGKCSVALLNETESVLSYLDKEDTFFYSLVYDPSLKTLLADKGEIRVGPRYQADIPEMLLEGESDEREQSKLEVKV
ELM Domain
SANT Domain
MTA1S WEAHNPLTDKQIDQFLVVARSVGTFARALDCSSSVRQPSLHMSAAAASRDITLFHAMDTLHKNIYDISKAISALVPQGGPVLCRDEMEEWSASEANLFEEALEKYGKDF
MTA1 WEAHNPLTDKQIDQFLVVARSVGTFARALDCSSSVRQPSLHMSAAAASRDITLFHAMDTLHKNIYDISKAISALVPQGGPVLCRDEMEEWSASEANLFEEALEKYGKDF
MTA2 WDPDNPLTDRQIDQFLVVARAVGTFARALDCSSSIRQPSLHMSAAAASRDITLFHAMDTLQRNGYDLAKAMSTLVPQGGPVLCRDEMEEWSASEAMLFEEALEKYGKDF
MTA3 WDPNSPLTDRQIDQFLVVARAVGTFARALDCSSSVRQPSLHMSAAAASRDITLFHAMDTLYRHSYDLSSAISVLVPLGGPVLCRDEMEEWSASEASLFEEALEKYGKDF
MTA3L WDPNSPLTDRQIDQFLVVARAVGTFARALDCSSSVRQPSLHMSAAAASRDITLFHAMDTLYRHSYDLSSAISVLVPLGGPVLCRDEMEEWSASEASLFEEALEKYGKDF
SANT Domain
MTA1S TDIQQDFLPWKSLTSIIEYYYMWKTTDRYVQQKRLKAAEAESKLKQVYIPNYNKPNPNQISVNNVKAGVVNGTG**************APGQSPGAGRACESCYTTQSY
MTA1 TDIQQDFLPWKSLTSIIEYYYMWKTTDRYVQQKRLKAAEAESKLKQVYIPNYNKPNPNQISVNNVKAGVVNGTG**************APGQSPGAGRACESCYMSSLR
MTA2 NDIRQDFLPWKSLASIVQFYYMWKTTDRYIQQKRLKAAEADSKLKQVYIPTYTKPNPNQIISVGSKPGM*NGAGFQKGLT********************CESCHTTQSA
MTA3 NDIRQDFLPWKSLTSIIEYYYMWKTTDRYVQQKRLKAAEAESKLKQVYIPTYSKPNPNQISTSNGKPGAVNGAV***GTTFQPQNPLL********GRACESCYATQSH
MTA3L NDIRQDFLPWKSLTSIIEYYYMWKTTDRYVQQKRLKAAEAESKLKQVYIPTYSKPNPNQISTSNGKPGAVNGAV***GTTFQPQNPLL********GRACESCYATQSH
Zinc Finger
MTA1S ILLDILEEIWWLENANPVRWREARTKPQ
MTA1 QWYSWGPPNMQCRLCASCWTYWKKYGGLKMPTR***LDGERPG*PNRSNMSPHGLPARSSGSP*********KFAMKTRQAFYLHTTKLTRIARRLCREILRPWHAARN
MTA2 QWYAWGPPNMQCRLCASCWIYWKKYGGLKTPTQ***LEGATRGTTEPHS*RGHLSRPEAQSLSPYTTSANRAKLLAKNRQTFLLQTTKLTRLARRMCRDLLQPRRAARR
MTA3 QWYSWGPPNMQCRLCAICWLYWKKYGGLKMPTQSEEEKLSPSPTTEDPRVRSHVSRQAMQGMPVRNTGSP**KSAVKTRQAFFLHTTYFTKFARQVCKNTLRLRQAARR
MTA3L QWYSWGPPNMQCRLCAICWLYWKKYGGLKMPTQSEEEKLSPSPTTEDPRVRSHVSRQAMQGMPVRNTGSP**KSAVKTRQAFFLHTTYFTKFARQVCKNTLRLRQAARR
Zinc Finger
MTA1 PYLPINSAAIKAECTARLPEASQSPLVLKQAVRKPLEAVLRYLETHPRPPKPDPVKSVSSVLSSLTPAKVAPVINNGSPTILGKRSYEQHNGVDGNMKKRLLMPSRGLA
MTA2 PYAPINANAIKAECSIRLPKAAKTPLKIHPLVRLPLATIVKDLVAQAPLKPKTPRGTKTPINRNQLSQNRGLGGIMVKRAYETMAGAGVPFSANGRPLASG********
MTA3 PFVAINYAAIRAECKMLLNS
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

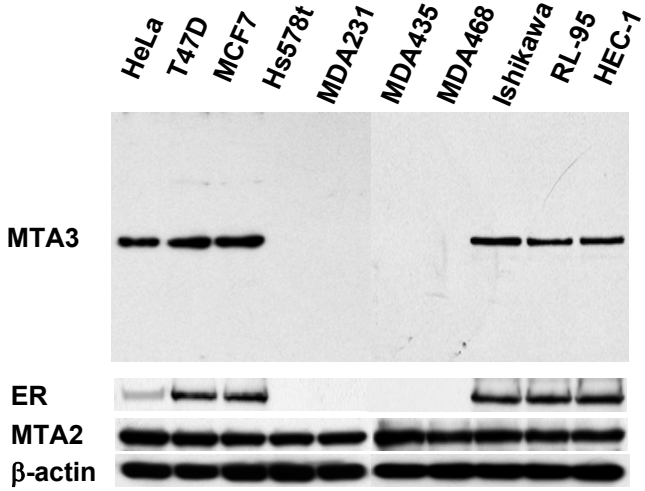
-HDAC presence implies gene regulation by modulation of histone acetylation

Mi-2/ NuRD complex in breast cancer

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

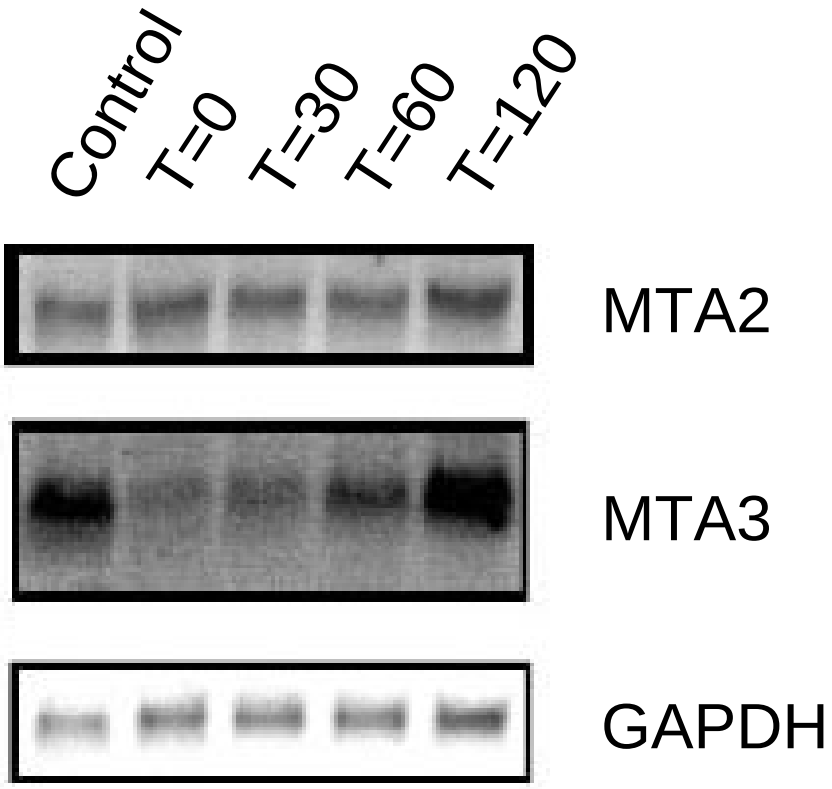
2. This complex is a component of a genetic regulatory circuit that impacts cell shape, physiology, and behavior

MTA3 expression correlates with ER status in human cell lines



Naoyuki Fujita

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

✗

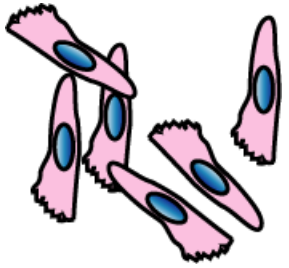
MTA3 OFF

✗

Transcriptional targets
On

↓

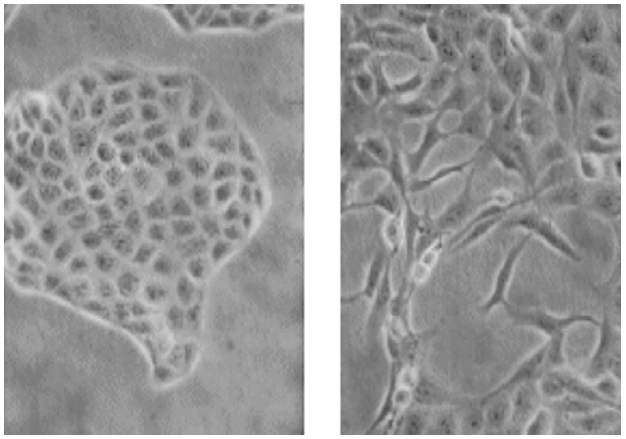
Functions???



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

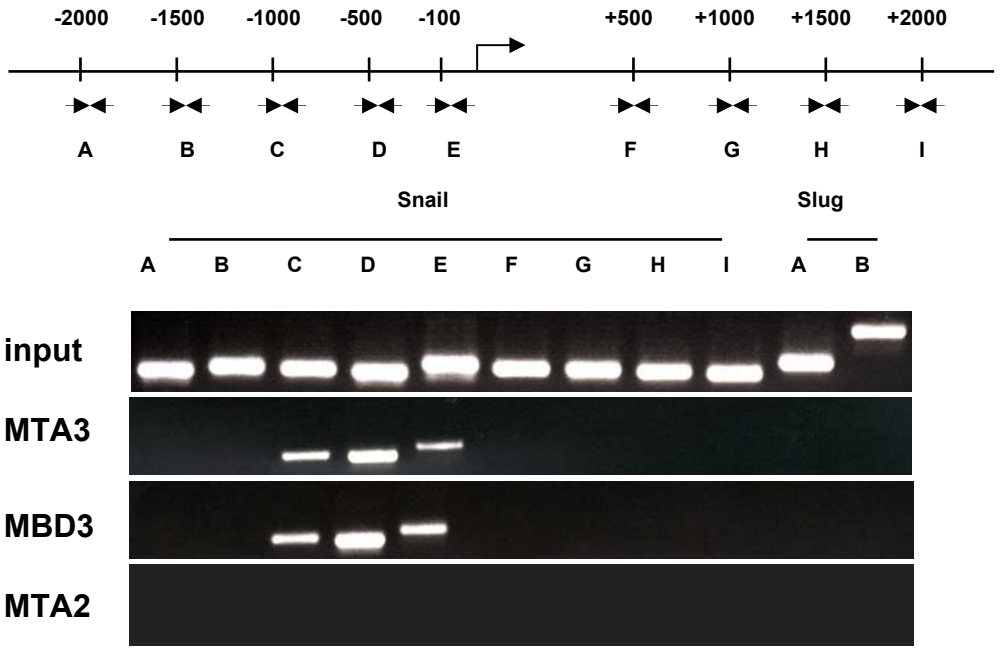
Snail – a candidate Mta3 target

- 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



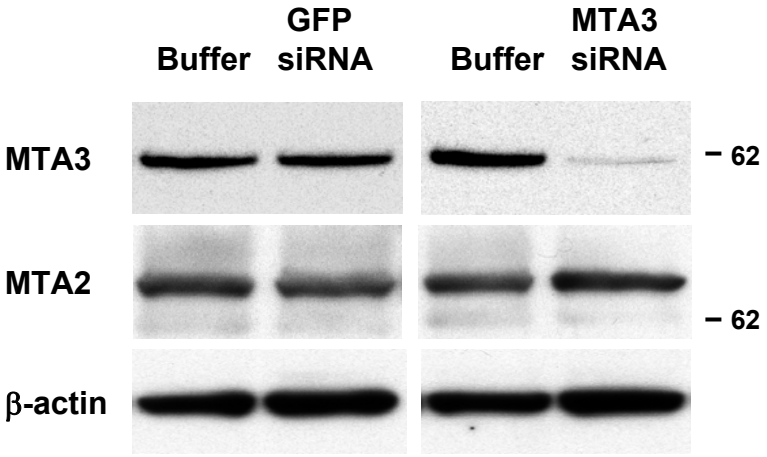
J Biol Chem. 2002 Oct 18;277(42):39209

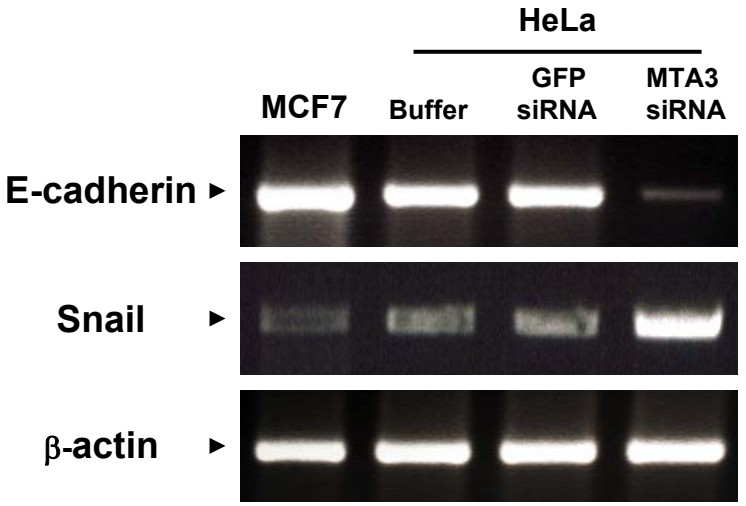
MTA3 localizes just upstream of the transcription start site at Snail



Naoyuki Fujita

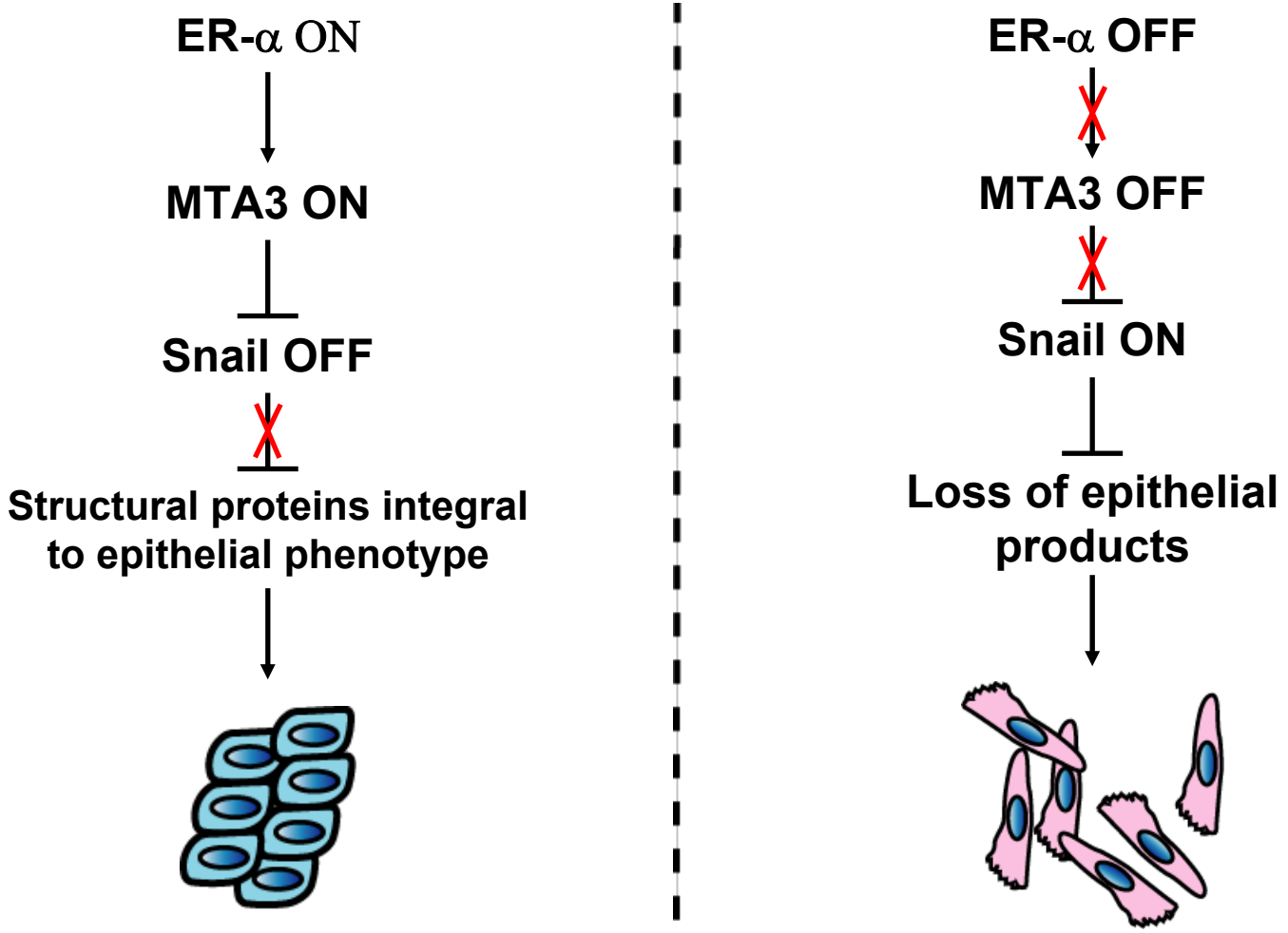
Depletion of MTA3 by RNAi leads to increased transcription of Snail





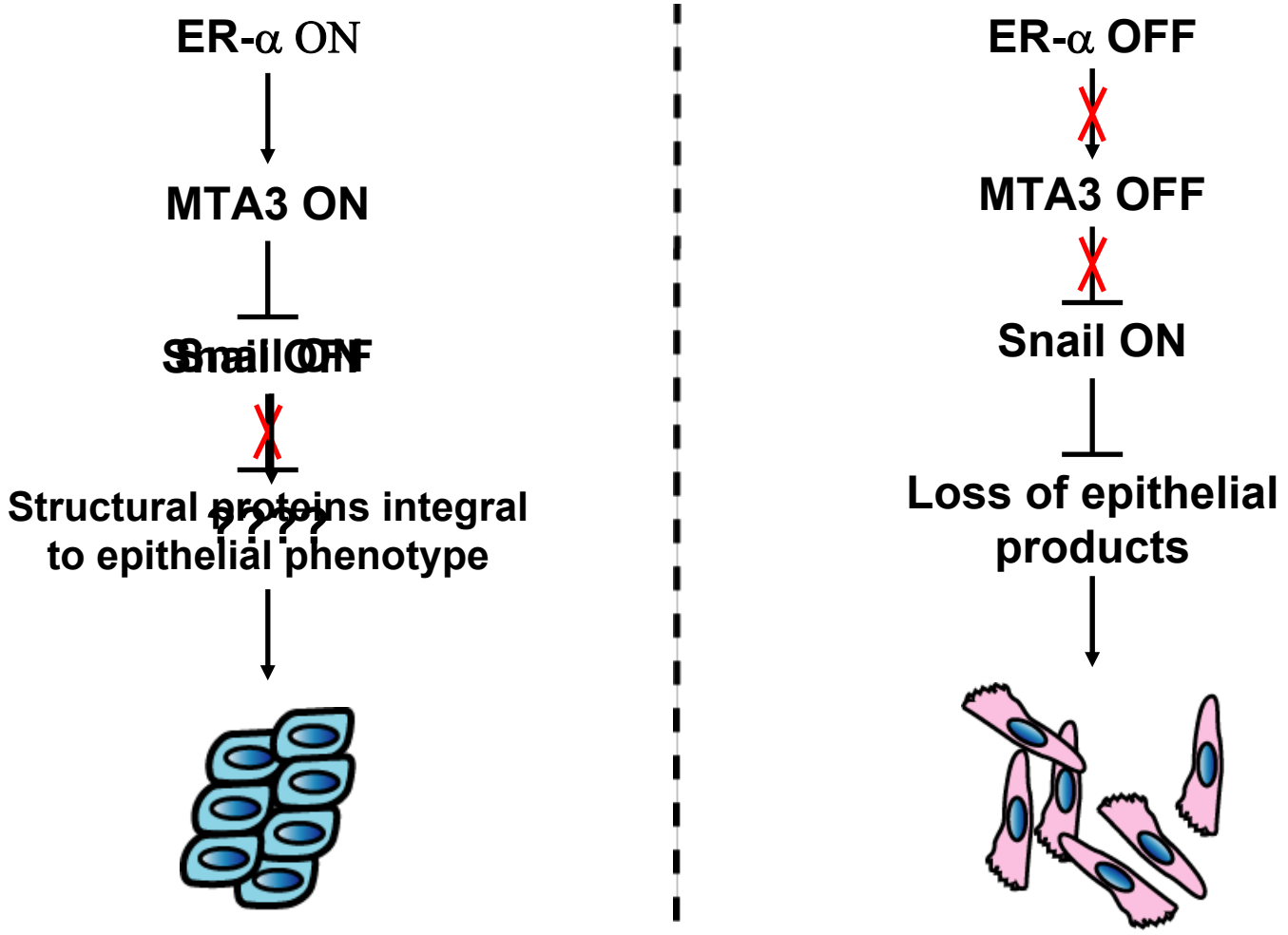
Naoyuki Fujita

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



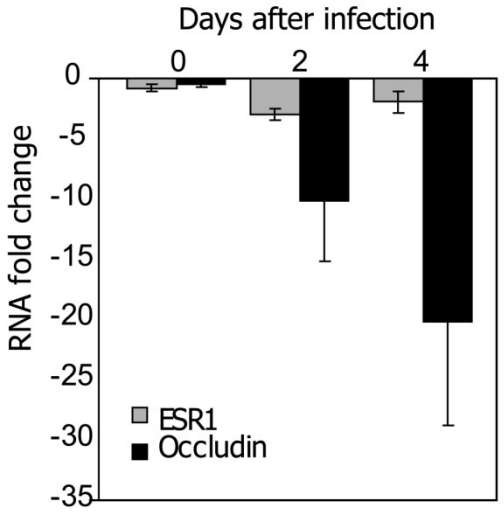
Fujita et al., 2003, Cell 113, 207-219
Fujita et al., 2004, Mol Endo 18, 2937-2949

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

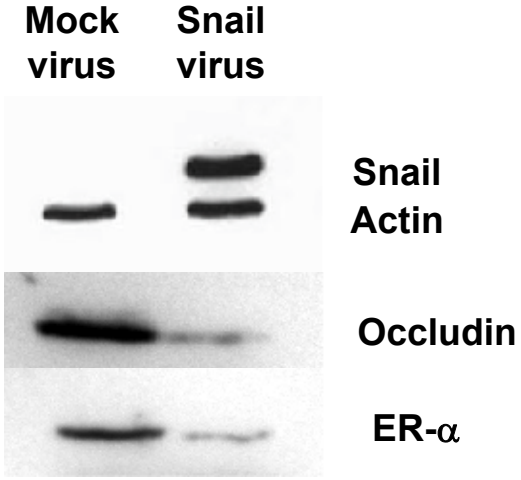


Aberrant Snail expression leads to loss of expression of epithelial products and ER-α

RT-PCR

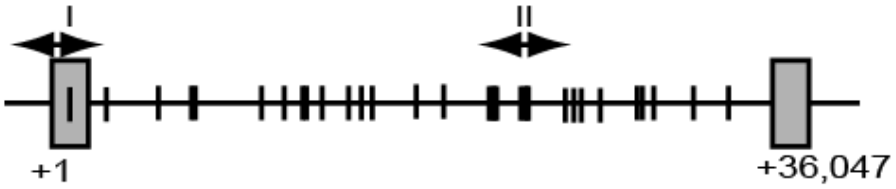


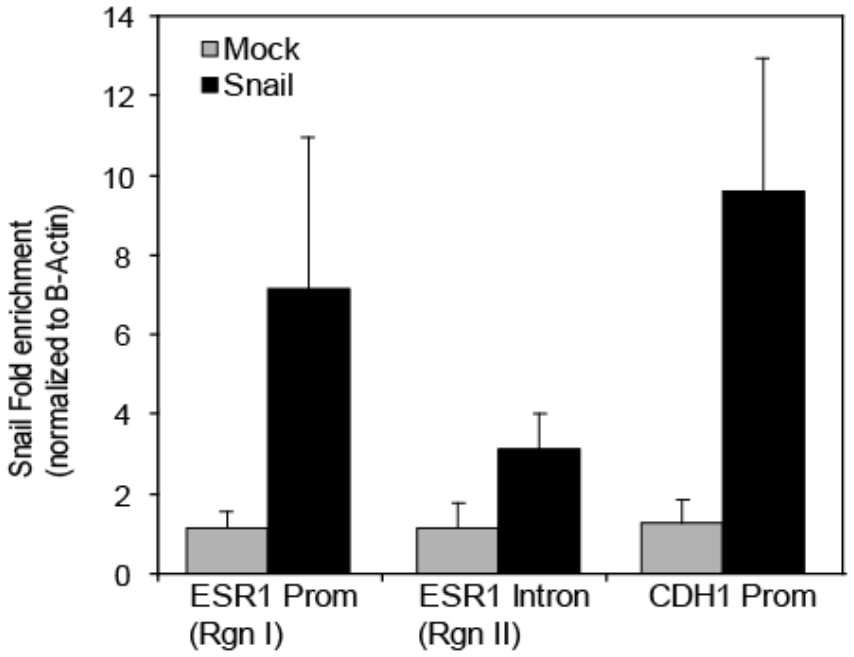
Immunoblot



Archana Dhasarathy

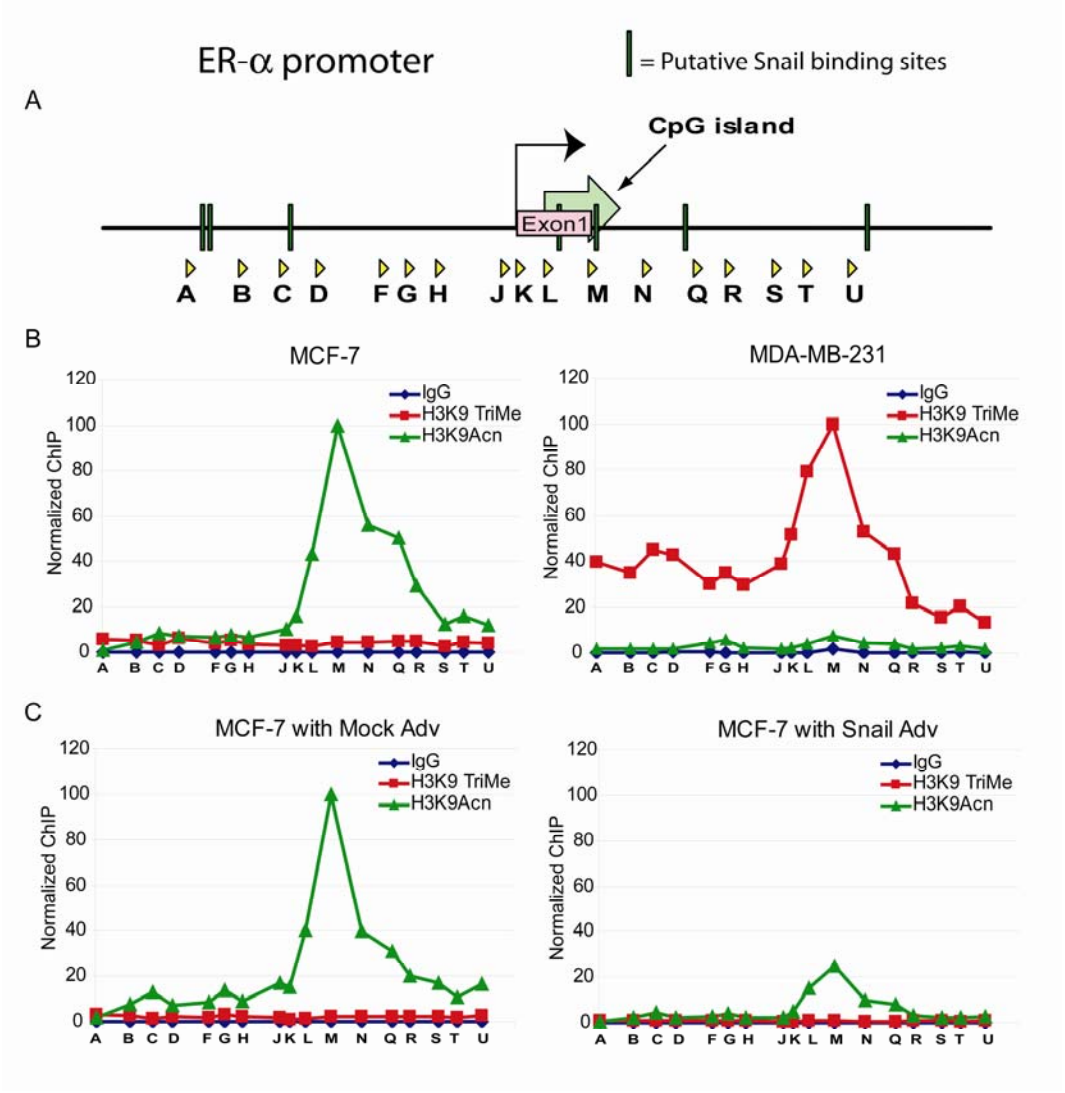
Snail directly binds the *ESR1* locus





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

- Aleksandra Adomas
- Archana Dhasarathy
- Anne Lai
- Chris Malone
- Jennifer Sims

NIEHS – Alumni

- Mehrnaz Fatemi
- Lisa Chadwick
- Sheri Denslow

Emory University

- Nathan Bowen
- Stuart Elgar
- Naoyuki Fujita
- Masahiro Kajita
- Matt Palmer
- Panya Taysavang