"Sperm DNA Packaging and Its Relationship to Function"

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How is Sperm Chromatin Organized?

Comparison with Somatic Cell Chromatin

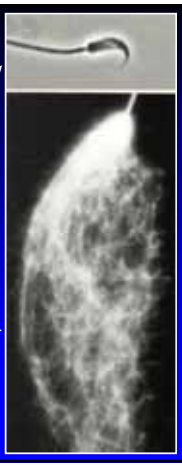
Advantage Packing DNA into a Chromosome

1 Chromosome = 1 Cassette

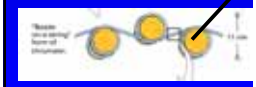
DNA Packaging

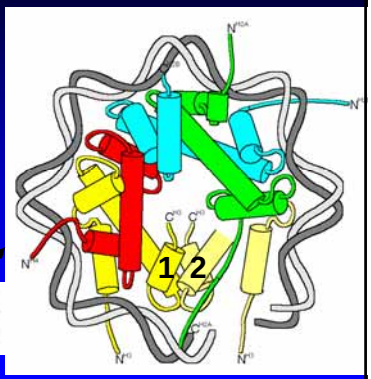
Phase micrograph of a hamster spermatozoon. All the DNA is packaged inside the flat, hooked shaped nucleus.

Fluorescent micrograph of a decondensed sperm cell shown at the **same magnification**. The DNA is stained with ethidium bromide.

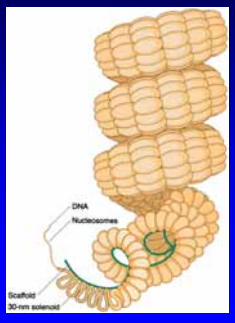


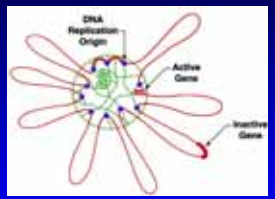
Histone Tetramer with DNA



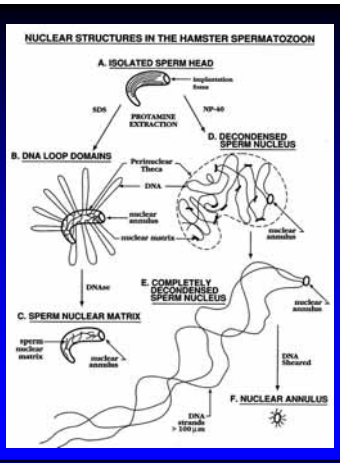


Chromosomal DNA is Organized into Functional Loop Domains by the Nuclear Matrix

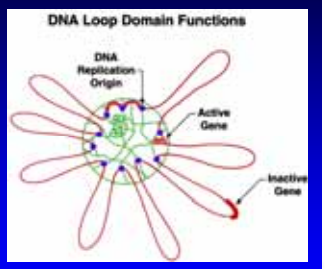




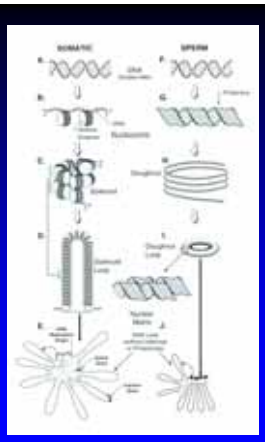
Mammalian Sperm Chromatin has Two Levels of Organization



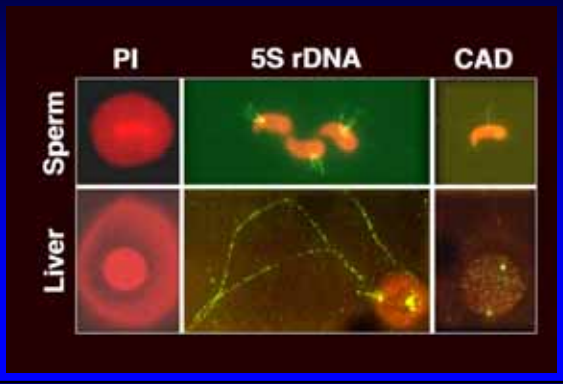
Sperm Chromatin is Packaged Differently than Somatic Cell DNA



The organization of the DNA into loop domains is the only similarity. →

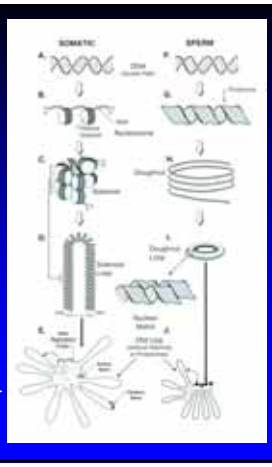


Visualization of DNA Loop Domains

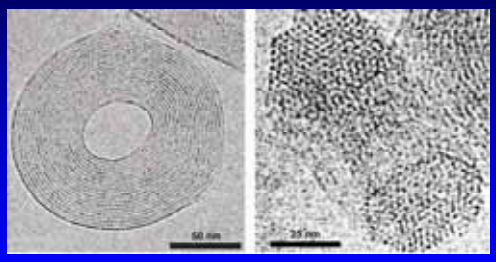


Donut-Loop Model for Protamine Binding

The organization of the DNA into loop domains is the only similarity.

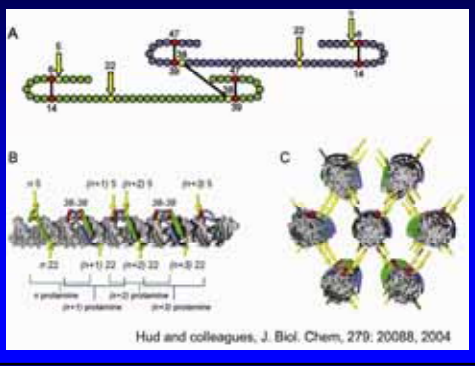


Synthetic Torids: Phage DNA + Co



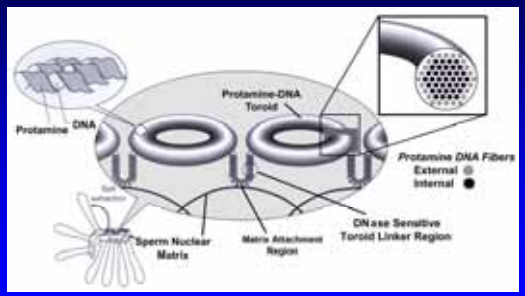
Hud and Downing, PNAS 98:14925, 2001

Model for Bull Protamine Binding to DNA

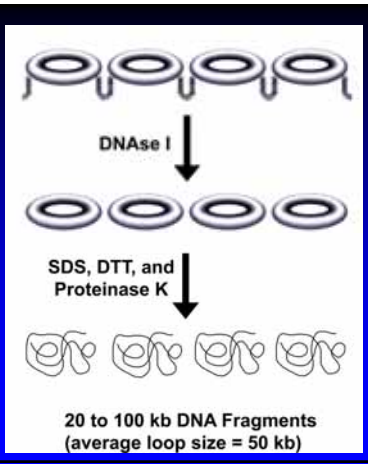


Hud and colleagues, J. Biol. Chem, 279: 20088, 2004

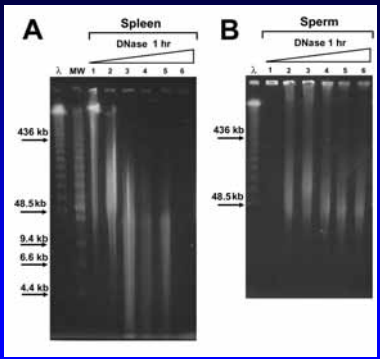
Donut-Loop Model for Sperm Chromatin



Model Predicts a Specific Pattern of DNAse Digestion

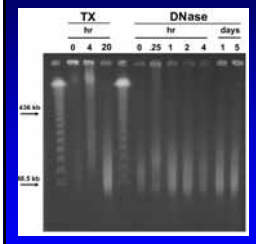


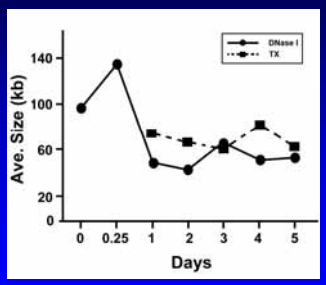
Hamster Sperm Chromatin is Less Sensitive to DNAse than Somatic Chromatin



Barbara Sotolongo

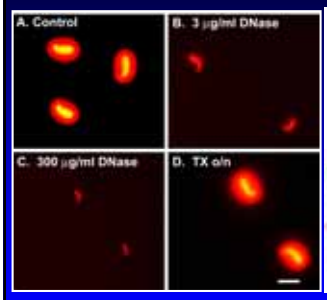
Comparison of Exogenous and Endogenous Nuclease Digestion of Sperm Chromatin

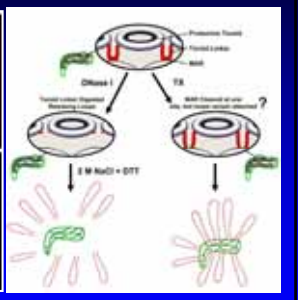




Barbara Sotolongo
Liz Lino

DNAse Releases DNA Loops from Sperm Nuclear Matrix





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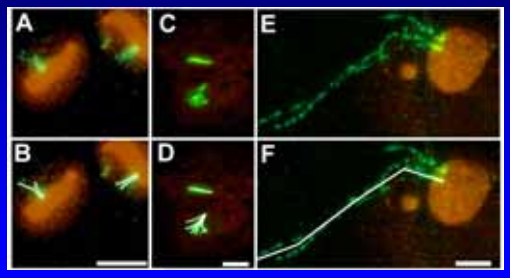
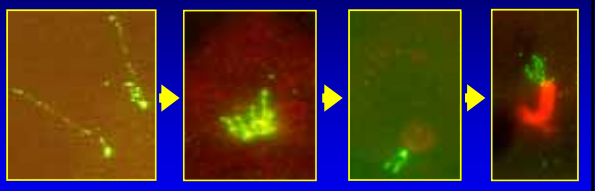
What about FUNCTION?:

Sperm Loop Domain Organization in Development

Quantitating DNA Loop Domain Size and Number

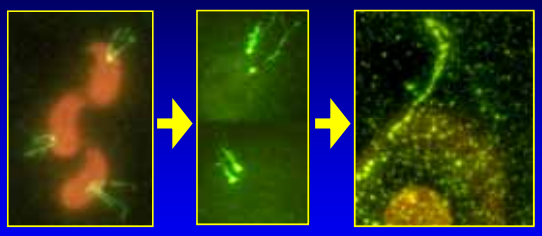


Changes in 5S rDNA Loop Structure During Spermatogenesis



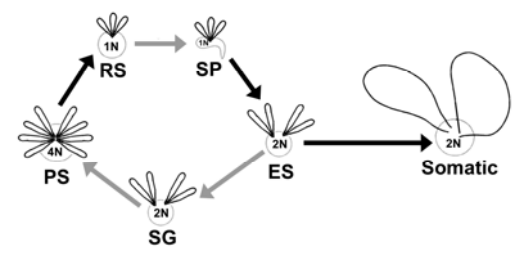
Spermatogonium | Pachytene Spermatocyte | Round Spermatid | Sperm -atozoon

Changes in 5S rDNA Loop Structure During Embryogenesis

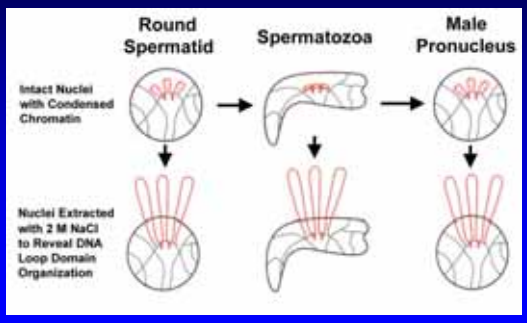


Spermatozoa | Embryonic Stem Cells | Liver

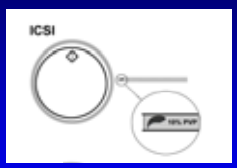
5S rDNA Loop Structure Changes



Hypothesized Role of Sperm Nuclear Matrix in Embryogenesis

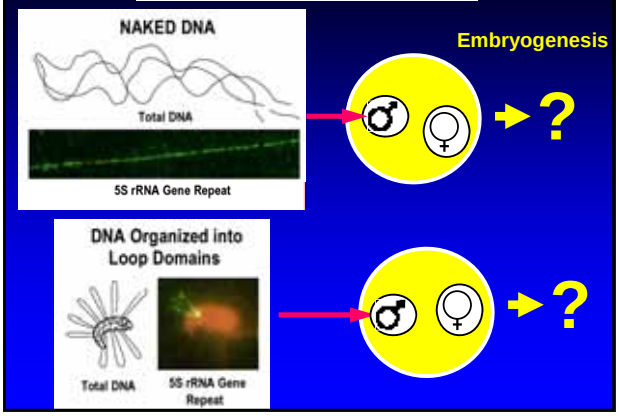


ICSI – The Biological Test of Any Model

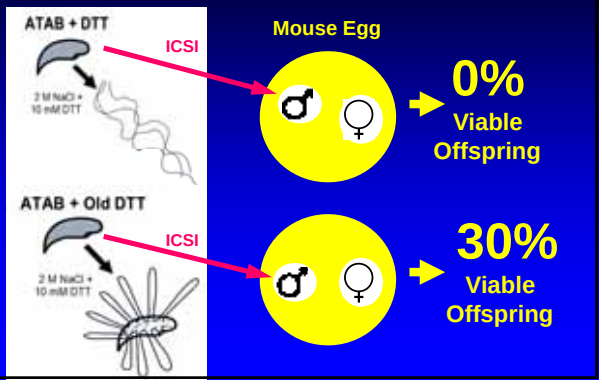


ICSI is an important advantage in study that the sperm model offers for studying chromatin structure.

Ideal Experiment

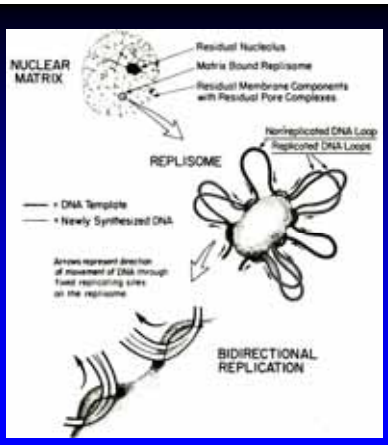


Results of ICSI Experiments

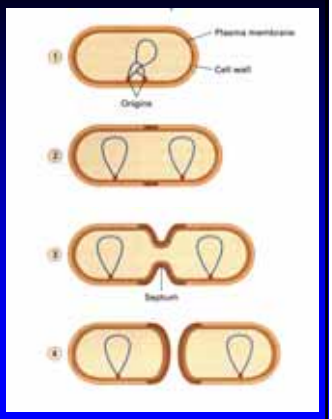


DNA is Synthesized at a Fixed Site

Pardoll, et al., Cell, 19:527, 1980



Visualization of DNA Replication in Bacteria

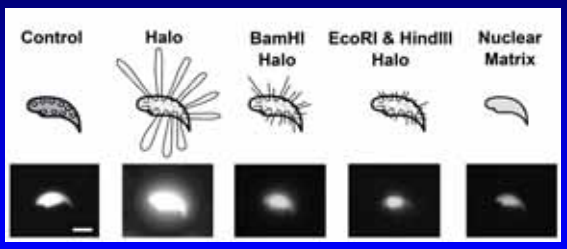


Lemon KP, Grossman AD.

"Localization of bacterial DNA polymerase: evidence for a factory model of replication."

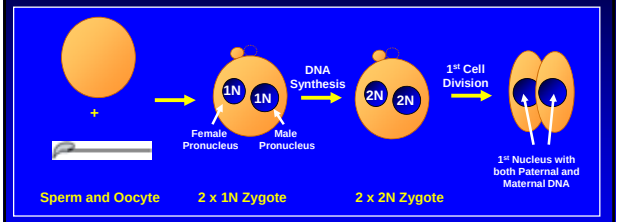
Science 282:1430-1

Nuclear Preparations Injected



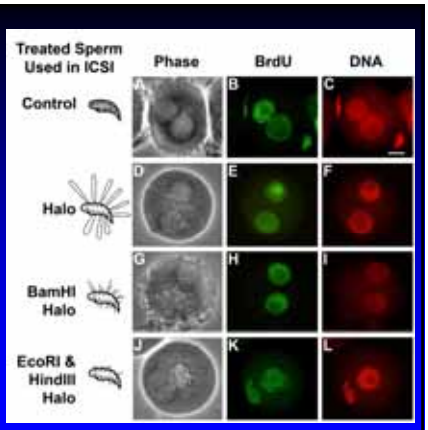
Jeff Shaman and Yasuhiro Yamauchi, *J. Cell. Biochem, in press, 2007*

Mammalian Fertilization



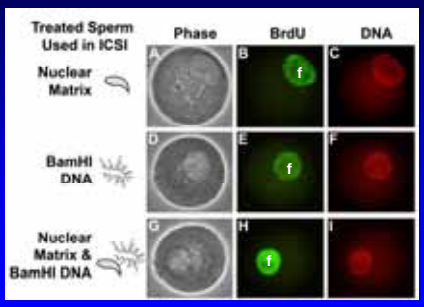
The Sperm and Oocyte DNA replicate *independently* in the first cell cycle of the embryo.

In Situ Nuclear Preps



Jeff Shaman and Yasuhiro Yamauchi, *J. Cell. Biochem, in press, 2007*

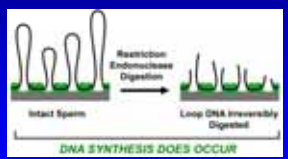
Reconstituted Nuclear Matrices



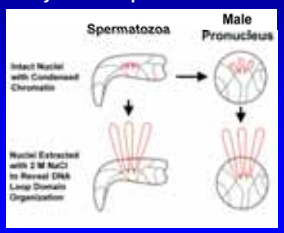
Jeff Shaman and Yasuhiro Yamauchi, *J. Cell. Biochem, in press, 2007*

Sperm MARs are Required for Paternal Pronuclear DNA Synthesis

1. Only intact MARs are necessary for DNA replication.



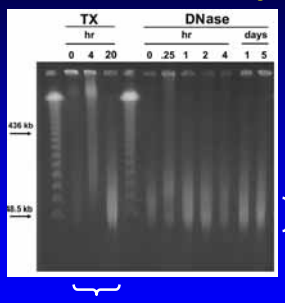
2. The matrix attachment sites in the sperm cell may be the same ones used by the male pronucleus



A Sperm Nuclease

Sperm Loop Domain Organization in DNA Degradation

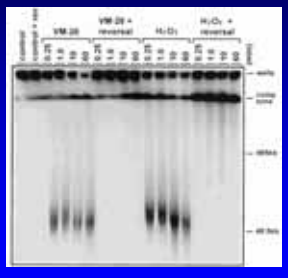
Defining the Endogenous Sperm Nuclease Activity

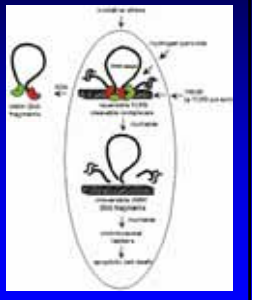


~ 50 kb, Loop-sized DNA fragments

Endogenous sperm chromatin digestion?

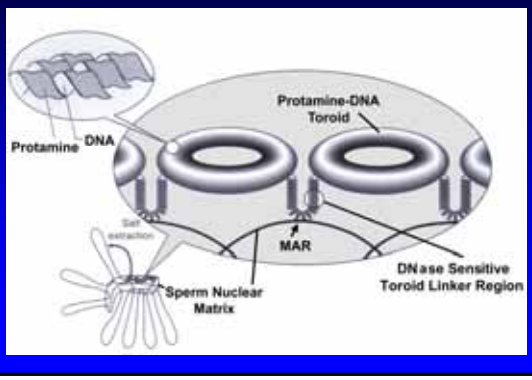
Loop Domains Function in Somatic Cell Apoptosis





Li, et al., Genes Dev. 13:1553, 1999

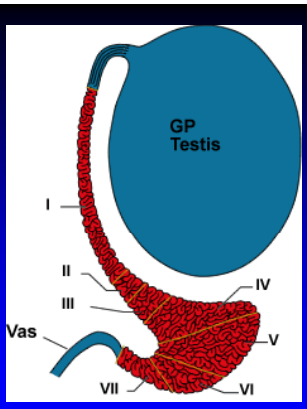
Donut-Loop Model for Sperm Chromatin



Sperm Matures in the Epididymis

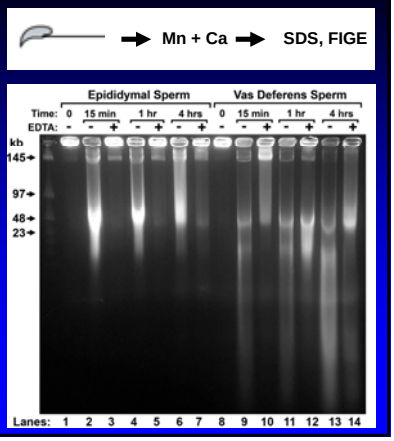
Caput Epididymis:
- Non Motile
- Cannot Fertilize

Cauda Epididymis:
- Motile
- Can Fertilize



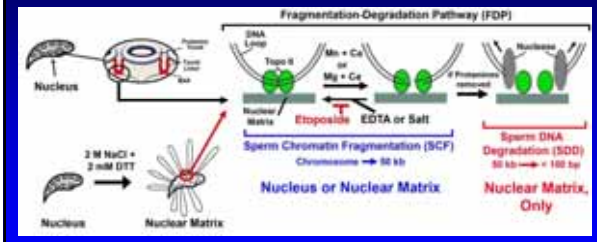
Gary Hunnicutt, PhD, Population Research Council
http://www.popcouncil.org/projects/BIO_SpermMaturation.html

Sperm Chromatin Fragmentation (SCF)

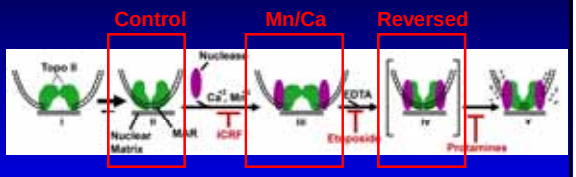


Yasuhiro Yamauchi, Jeff Shaman, and Segal Boaz, *Biol. Reprod. 77:407-15, 2007*

Model for Sperm Chromatin Fragmentation (SCF)

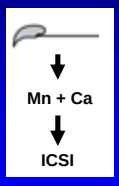


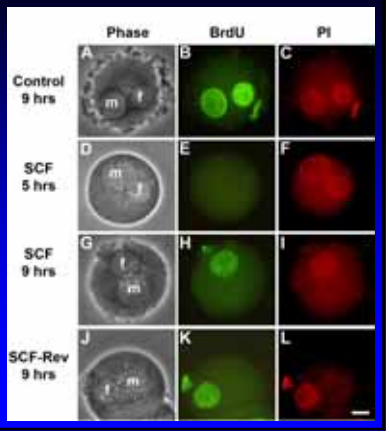
ICSI – The Biological Test of the Model



Development to Blastocyst after ICS	85.2%	7.4%	16.0%

Paternal DNA is Degraded 6 hrs After SCF Sperm are Injected

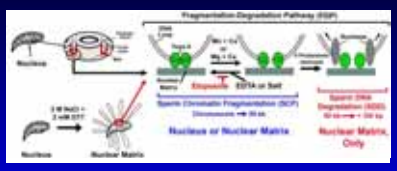




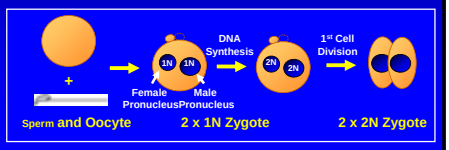
Yasuhiro Yamauchi and Jeff Shaman, *Biol. Reprod. 76:666, 2007*

Two Hints for Paternal DNA Degradation

1. Starts at MAR, like DNA Replication



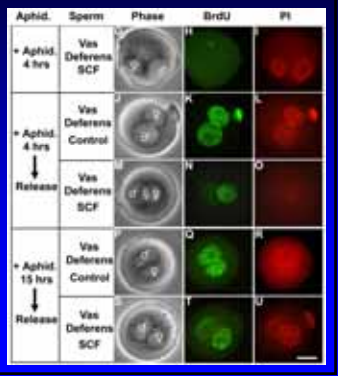
2. Does not start until DNA synthesis begins



DNA Intact → DNA Degraded

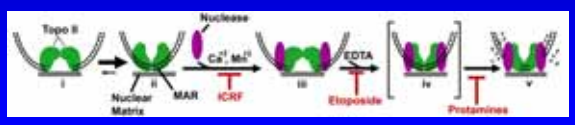
DNA Degradation is Dependent on DNA Synthesis

1. Aphid. prevents SCF-induced DNA Degradation
2. Release from Aphid. allows SCF-induced DNA degradation to proceed.
3. Prolonged time in Aphid. reverses DNA degration



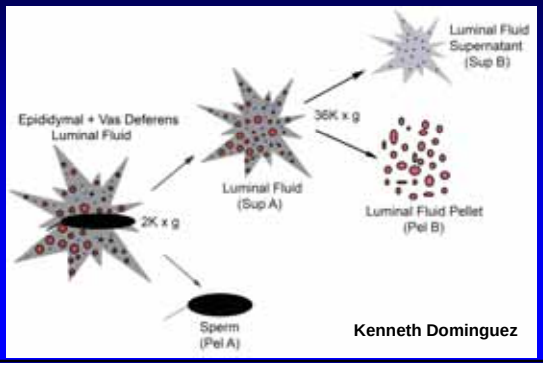
Yasuhiro Yamauchi, Jeff Shaman, and Segal Boaz, *Biol. Reprod. 77:407-15, 2007*

Sperm Nuclease is ACTIVATED by EGTA + Ca^{+2}



Fractionation of Vas Deferens Luminal Fluid

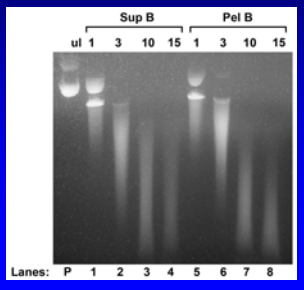
Vas Deferens contains vesicles that contain nuclease



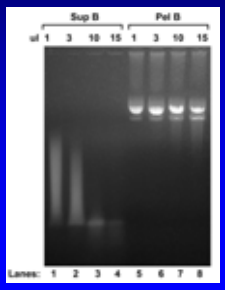
Kenneth Dominguez

Evidence that Nuclease is in Vesicles

Sup A → Centrifuge → Sup B + TX, Pel B + TX

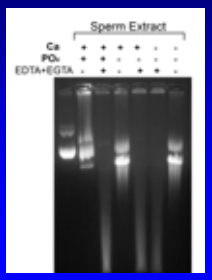


Sup A + TX → Centrifuge → Sup B, Pel B

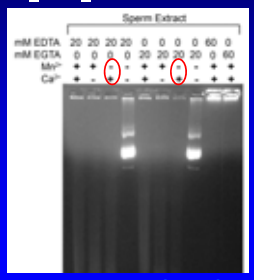


Sperm Nuclease Can be Activated by EDTA or EGTA

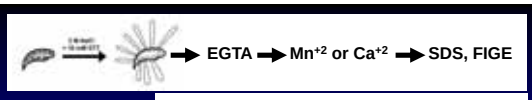
Extract ± $CaPO_4$ → Mn^{+2} + Ca^{+2} + Plasmid



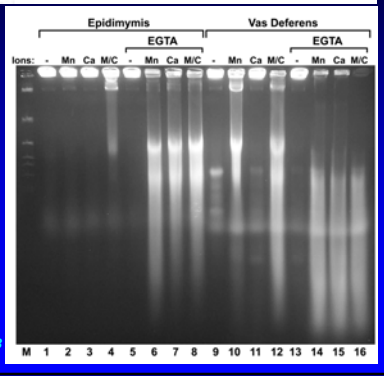
Extract ± EG/DTA → ±Mn^{+2} ± Ca^{+2} + Plasmid



Kenneth Dominguez

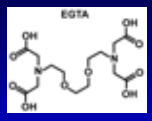


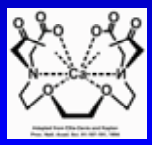
EGTA Activated Sperm DNA Degradation (SDD)

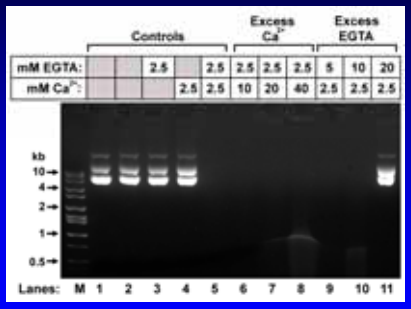


Segal Boaz, Ken Dominguez, and Jeff Shaman, *J. Cell. Biochem. 103:1636-1645, 2008*

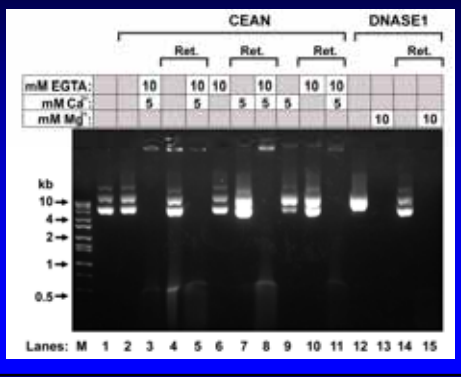
Chelated EGTA Activates EAN



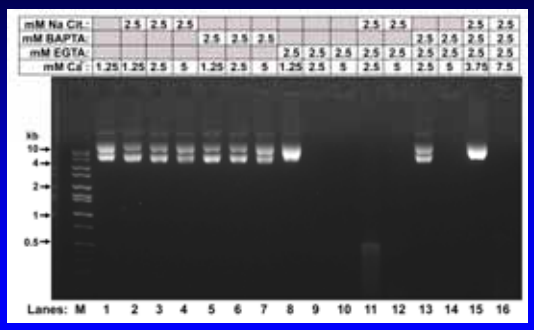




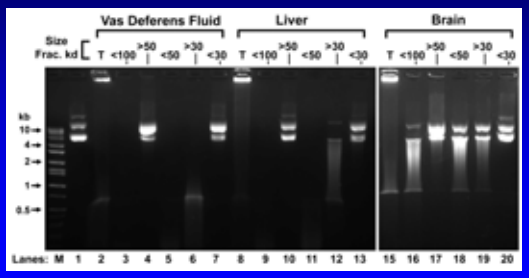
EGTA-Ca Activation is Reversible



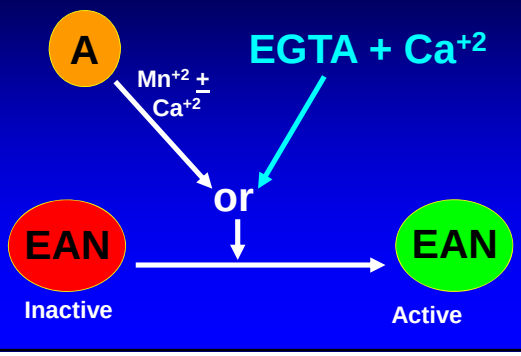
Other Chelators Do Not Activate Nuclease



EGTA-Ca Activated Nuclease is In Liver



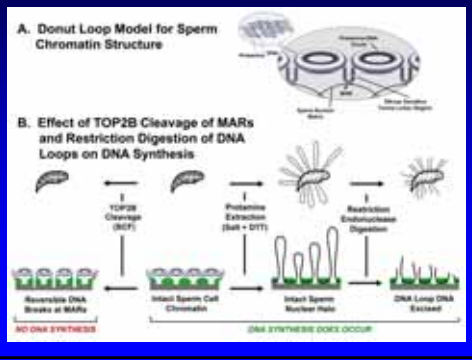
Model for EGTA Activation of EAN (EGTA Activated Nuclease)



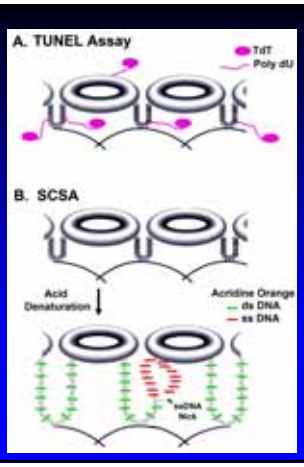
Tying it All Together:

Conclusion

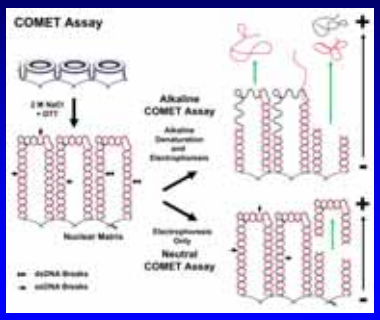
For DNA Replication/Degradation Toroid Linker/MAR is the Key Chromatin Structure



TUNEL and SCSA would be Expected to be Limited to Toroid-Linker or Nuclear Matrix Attachment Sites



Comet Assay Examines DNA Structure Independent of Chromatin Folding



Summary

For the preservation of spermatozoa for human ART, the most important component of sperm chromatin may be the Matrix Attachment Region, or MAR.