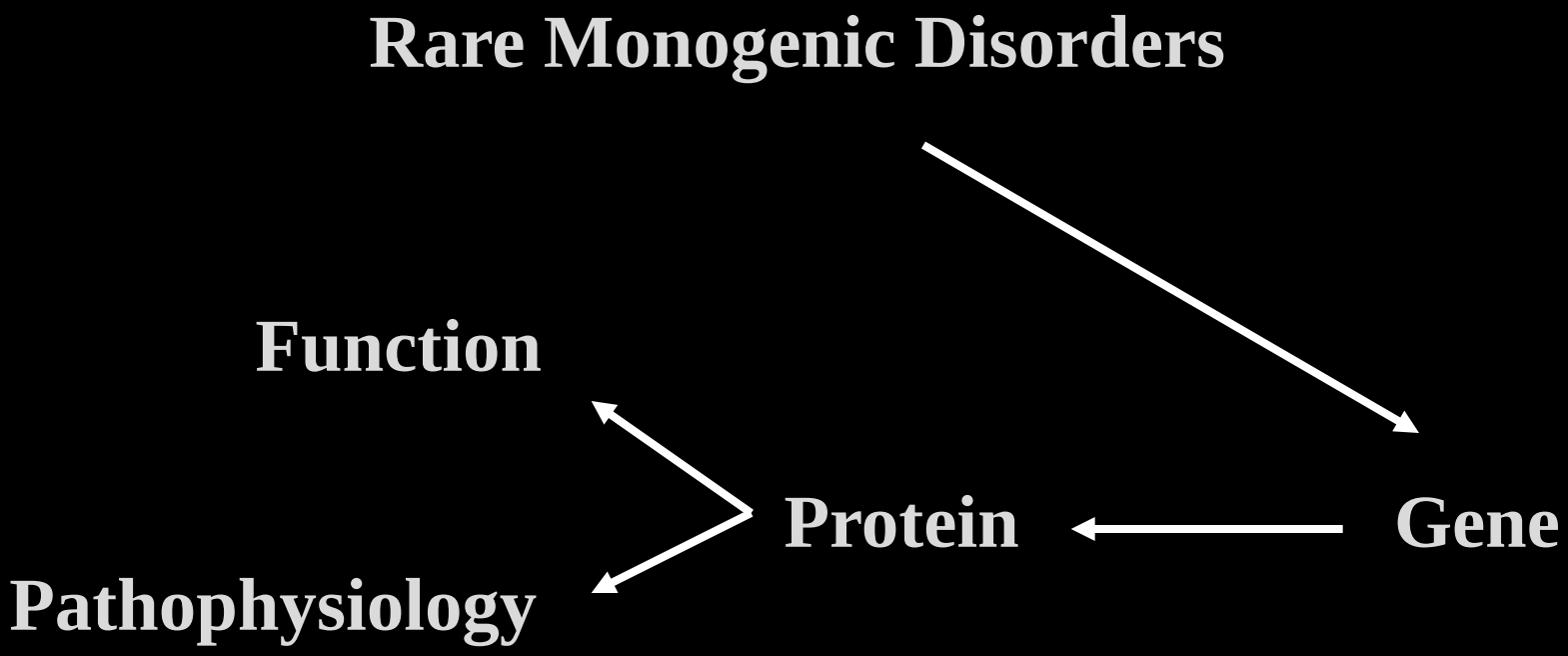

Rare Monogenic Disorders
Function
Protein
Gene
Pathophysiology

Episodic Nervous System Diseases

Migraine

Epilepsy

Periodic Paralysis

LQTS

Episodic Ataxia

Paroxysmal Dyskinesias

Phenotypes

Muscle diseases—periodic paralses

NMJ—Congential myasthenic, Lambert-Eaton

Peripheral nerve—pain syndromes

CNS—cortex

- **Migraine**
- **Epilepsy**
- **Familial Cortical Myoclonus**

CNS—striatum; Paroxysmal Dyskinesias

CNS—Cerebellum; Episodic ataxia

Similarities Among These Disorders

Episodic

Precipitating Factors

Stress, Fatigue, Dietary Factors, Alcohol, Caffeine

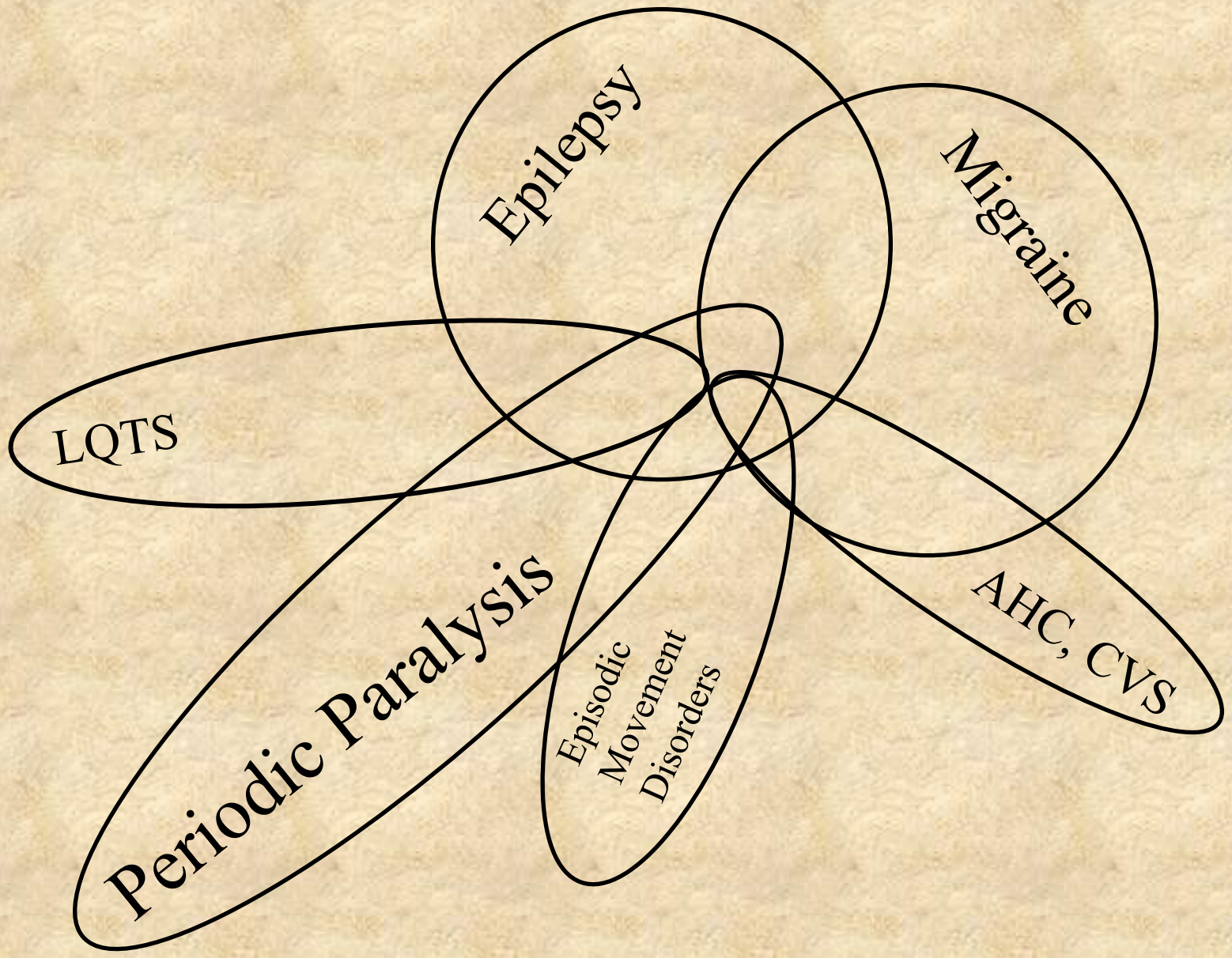
Therapeutic Responses

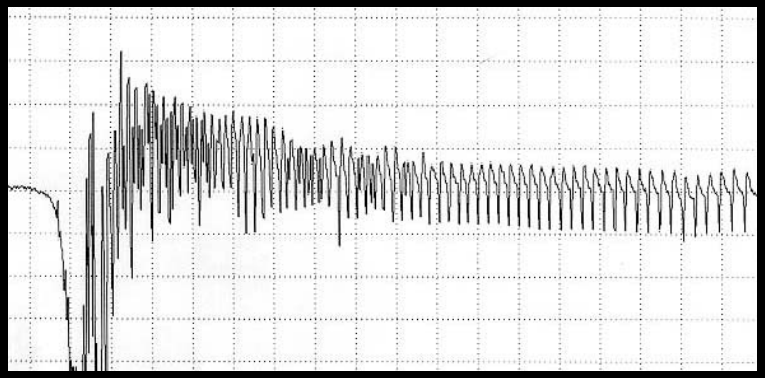
eg. CAI, Tegretol, Mexilitine

Hormonal Factors

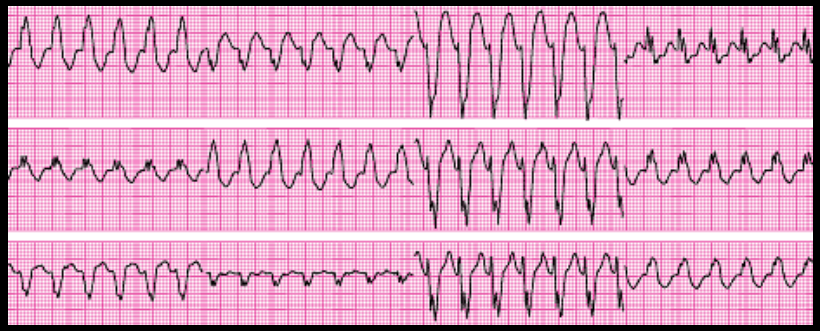
Attacks Frequently Decrease in Middle Age

Episodic Disorders

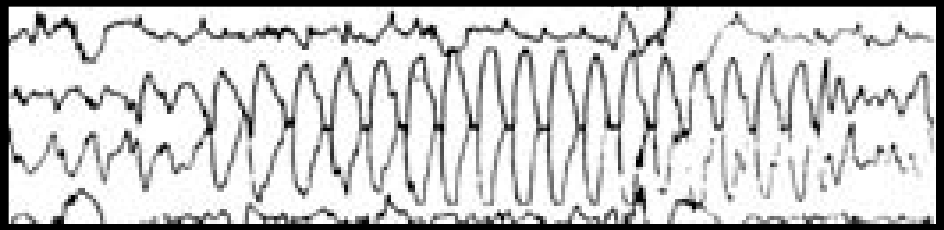




Myotonia



V-tach



EEG Seizure

Cell, Vol. 67, 1021–1027, November 29, 1991, Copyright © 1991 by Cell Press

Identification of a Mutation in the Gene Causing Hyperkalemic Periodic Paralysis

Louis J. Ptáček,*† Alfred L. George, Jr.,‡ Robert C. Griggs,§ Rabi Tawil,§ Roland G. Kallen,‡‖ Robert L. Barchi,‡# Margaret Robertson,†+ and Mark F. Leppert†+

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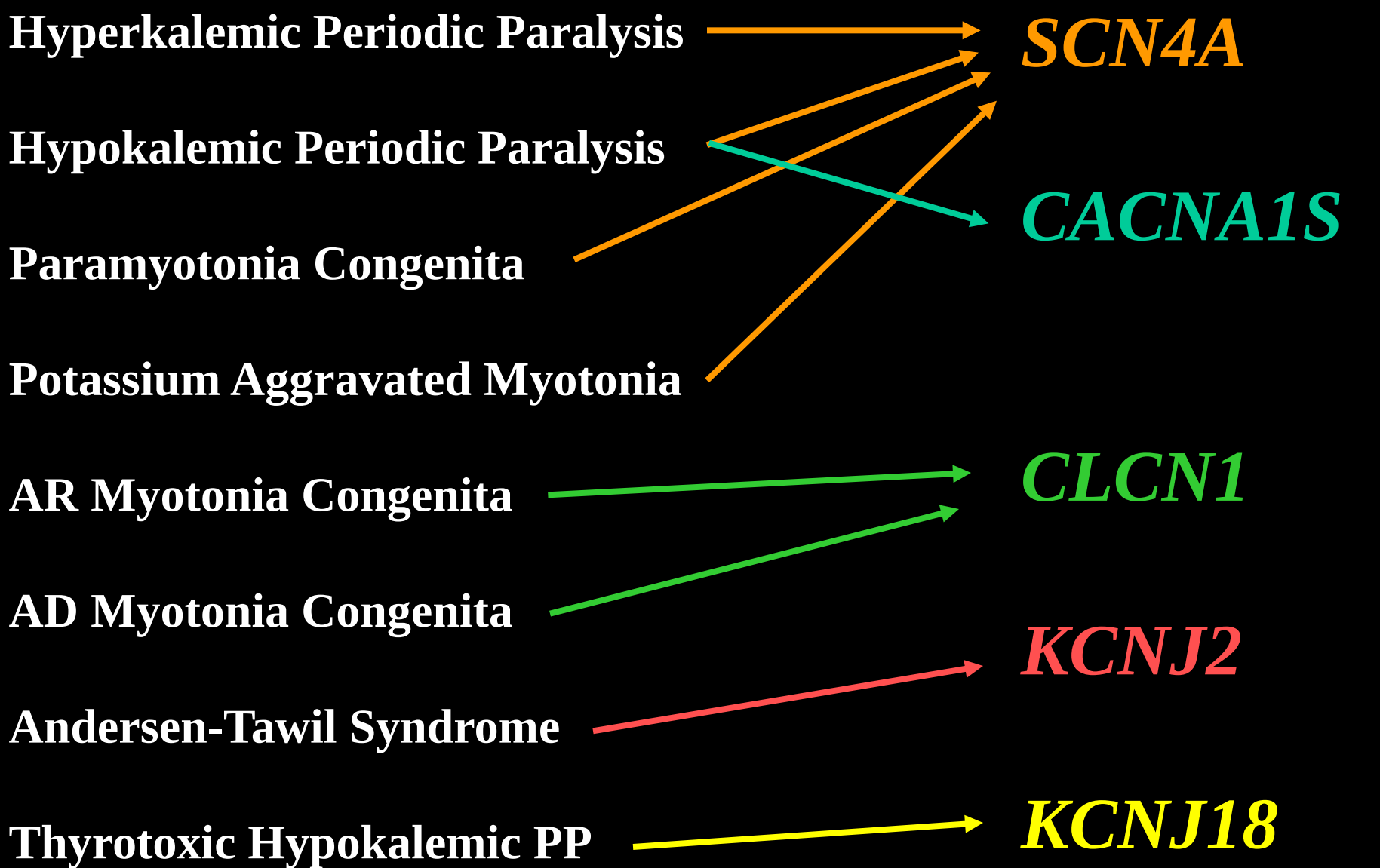
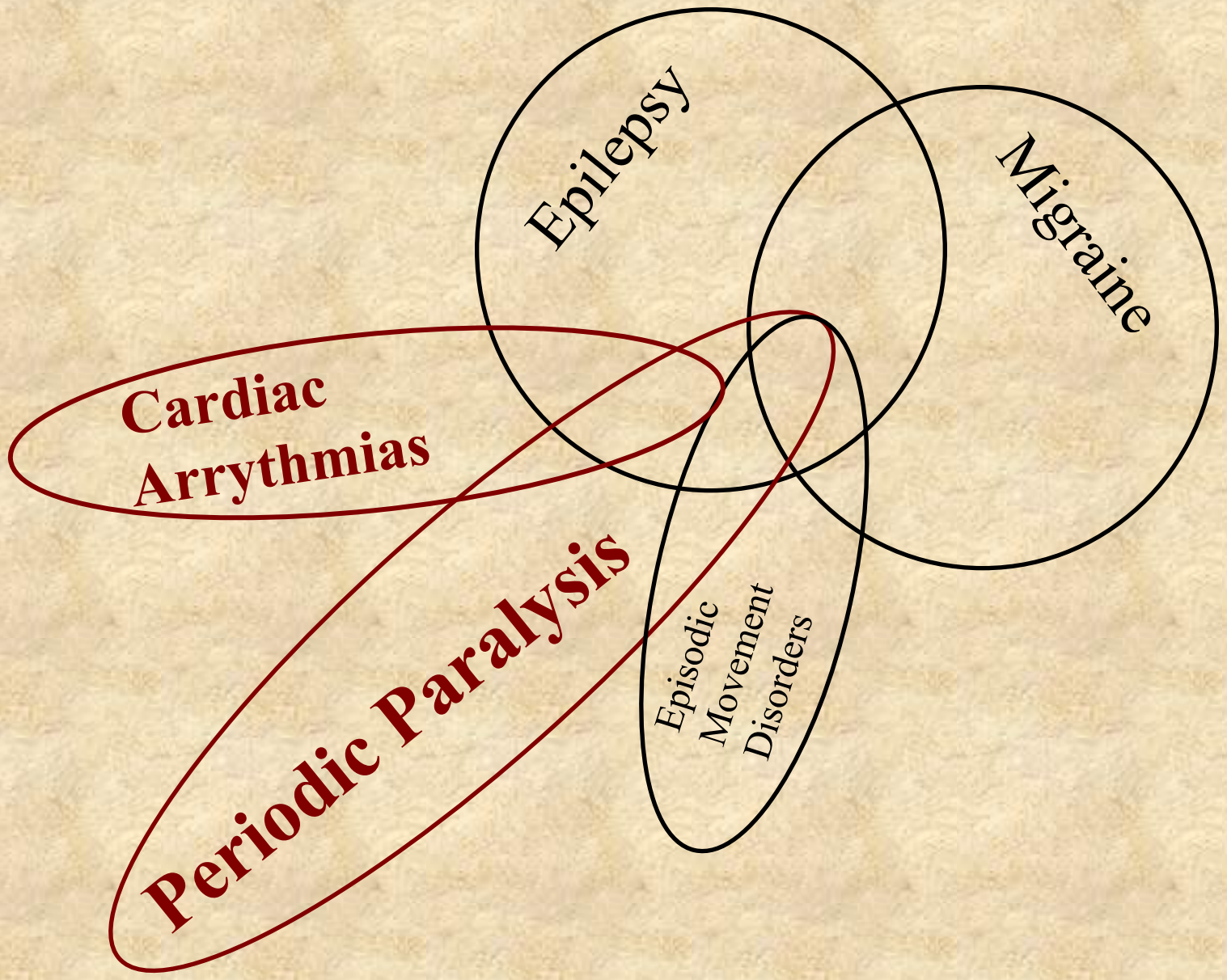
sion system would then allow analysis of ion channel response to pharmacologic interventions. Understanding HYPP and related disease states will lead to better understanding of normal muscle and other excitable tissues. Furthermore, the periodic paralyses and nondystrophic myotonic disorders may serve as a useful paradigm for studying other disorders in which abnormalities in electrical properties of cell membranes are known to be important. Such diseases include epilepsy, peripheral nerve disorders, and cardiac dysrhythmias.

•*Ptáček et al., Cell 1991*

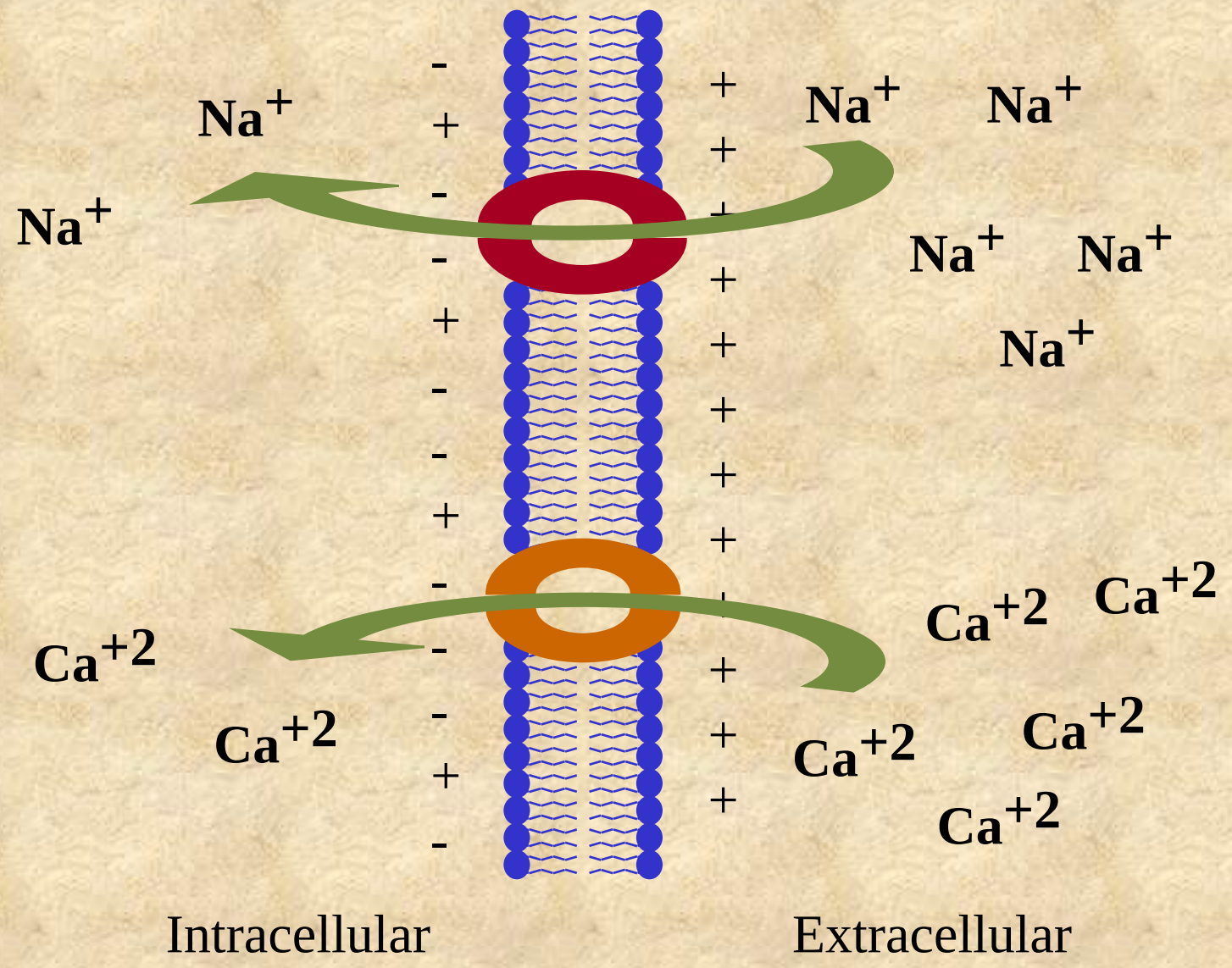
Non-dystrophic Myotonia/Periodic Paralysis



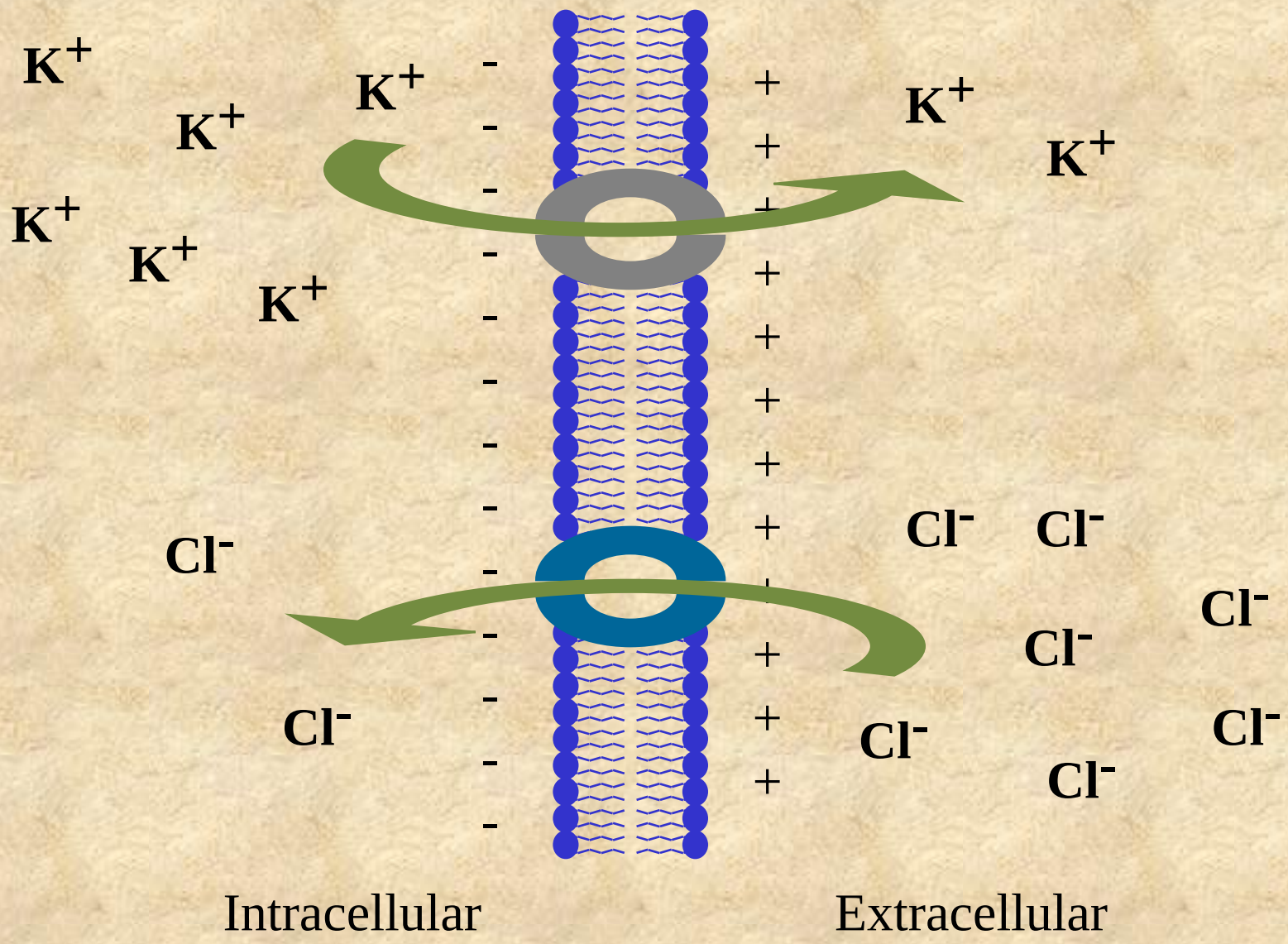
Andersen/Tawil Syndrome



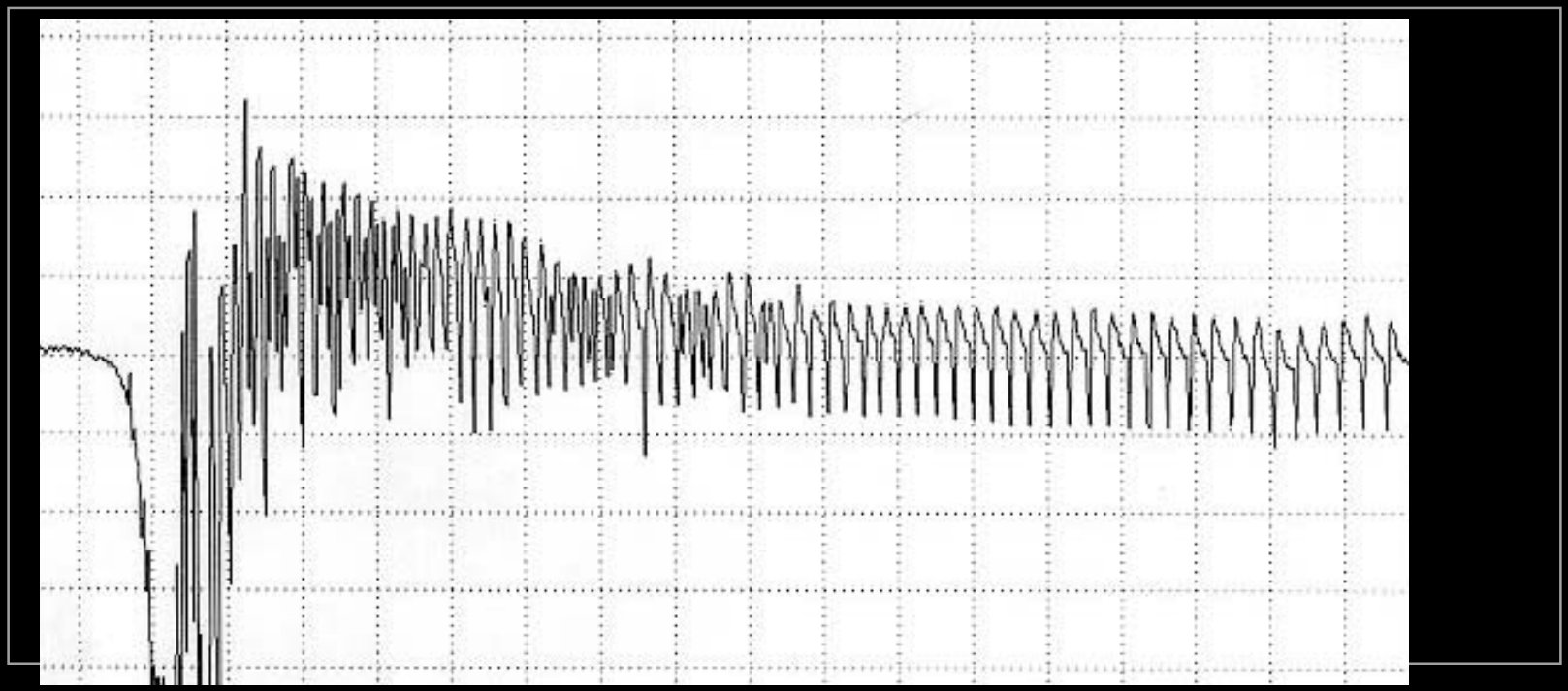
Depolarizing Effects



Polarizing or Re-polarizing Effects



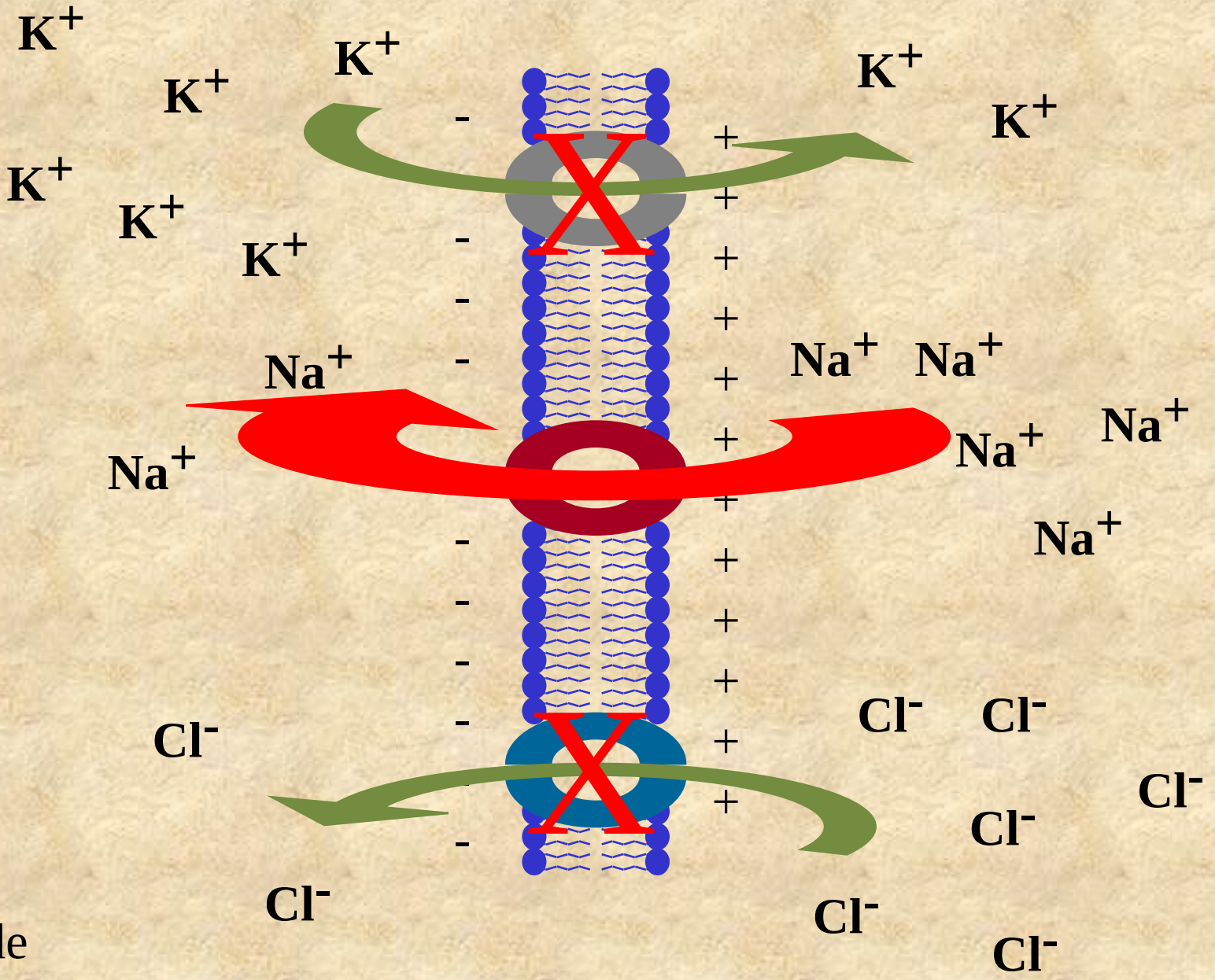
Myotonia



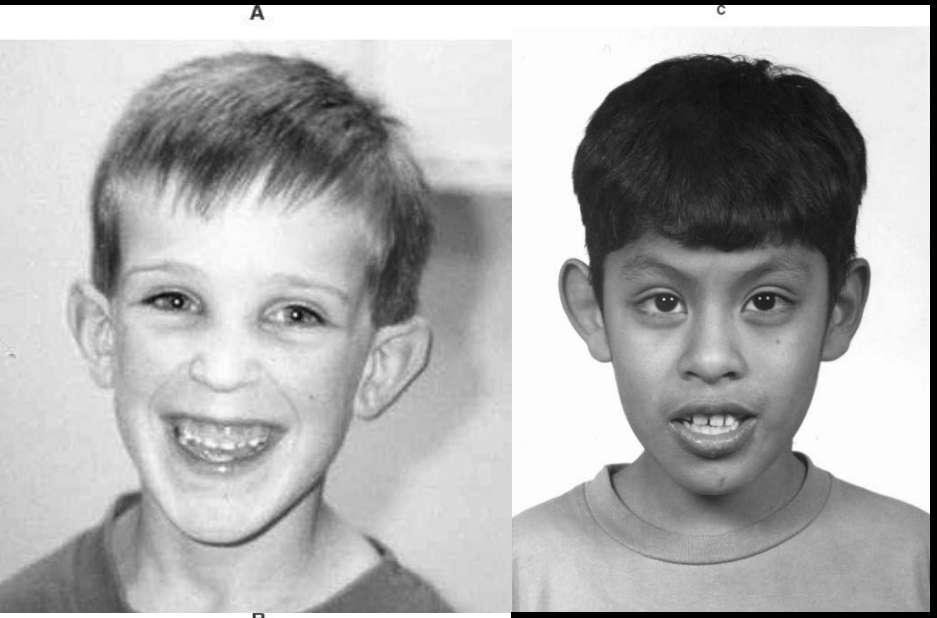
Myotonia Congenita

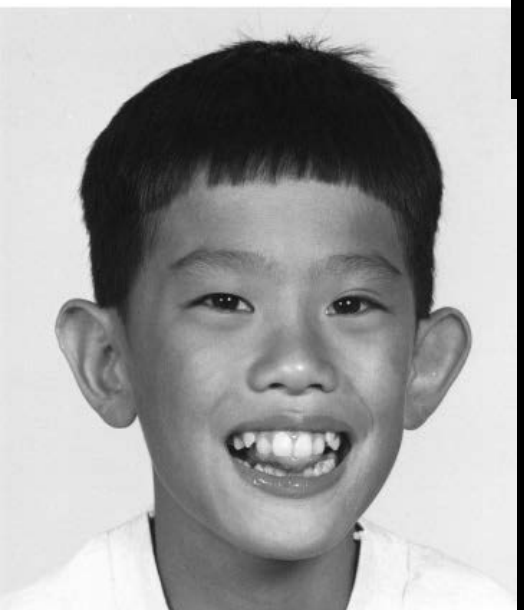
- Goat model of disease—decreased g_{Cl}
- Physiologic study of explanted patient muscle showed decreased g_{Cl}
- Mapping of MC locus to chromosome 7 near *ClC-1*
- Positional--candidate approach

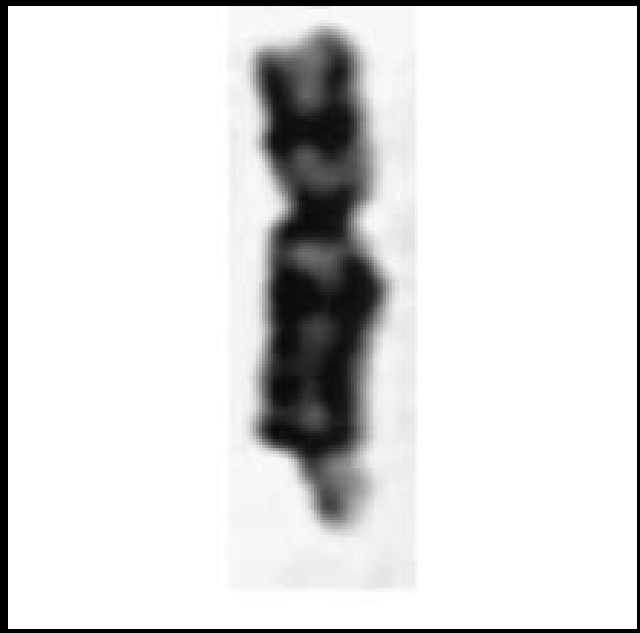
Muscle Membrane Hyperexcitability



Fragile X mental retardation



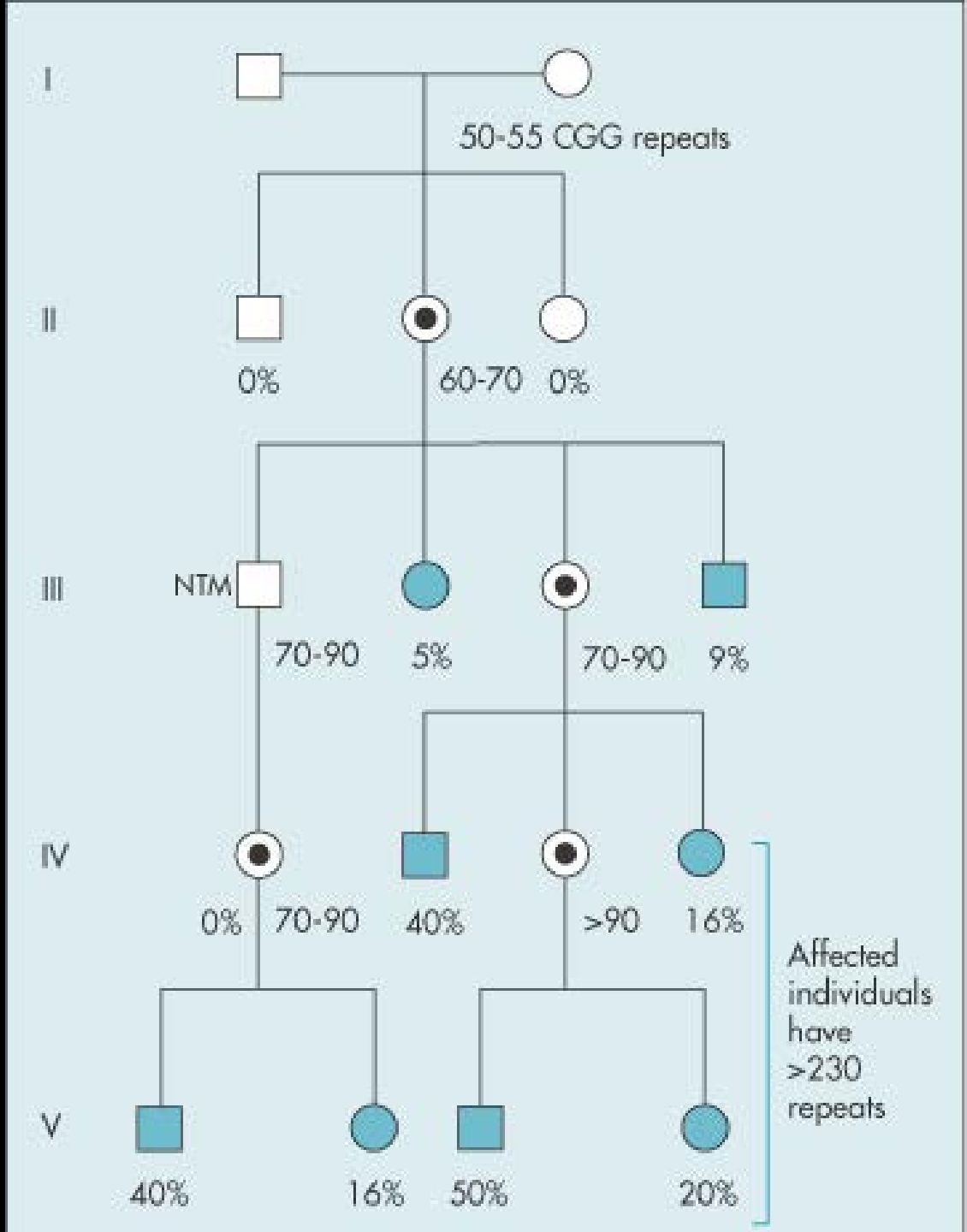


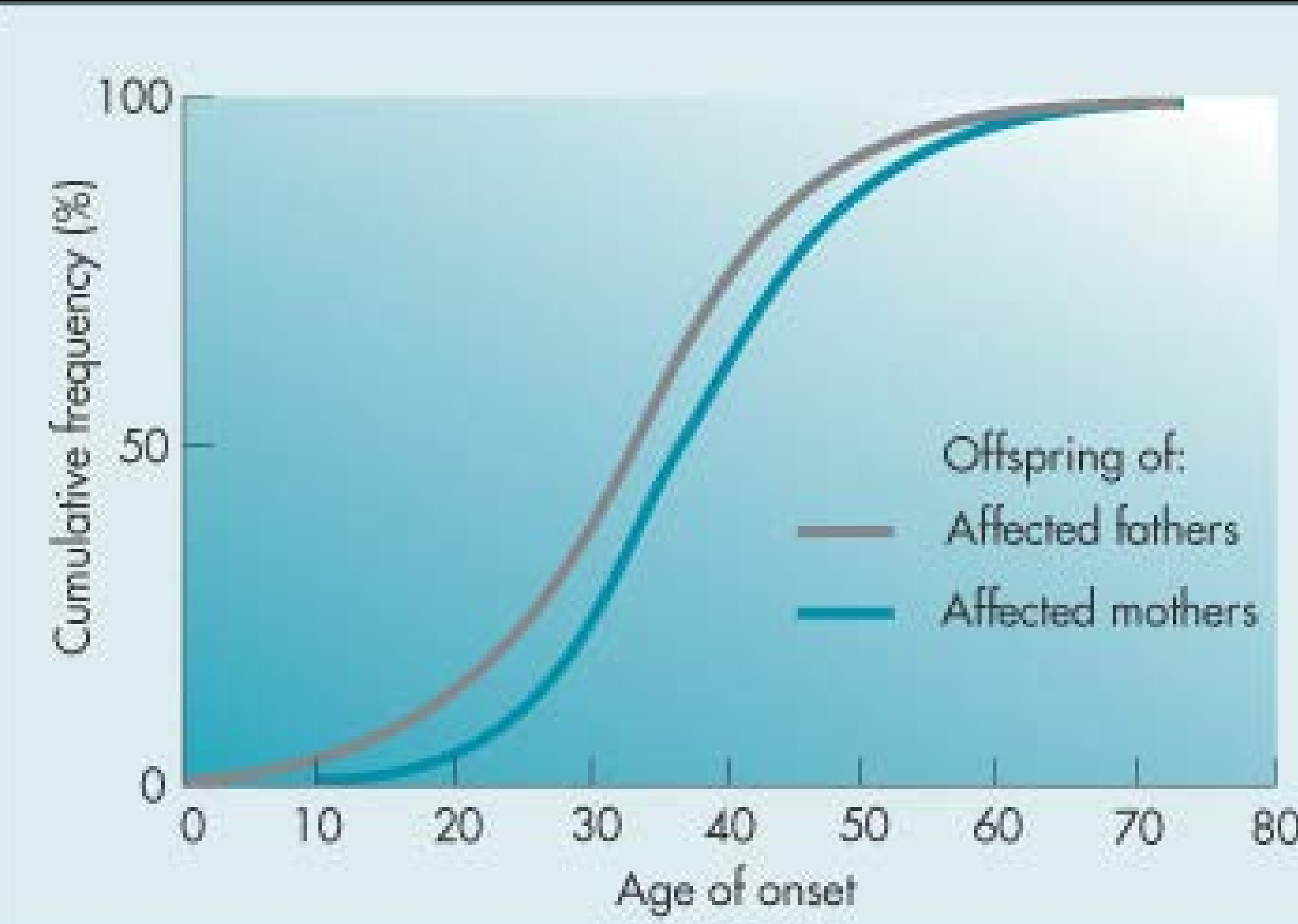


Anticipation

- A phenomenon where an unstable mutation segregates in families such that, *on average*, disease severity increases as the mutation is passed through subsequent generations

The Sherman Paradox




100
50
0
Cumulative frequency (%)
0
10
20
30
40
50
60
70
80
Age of onset
Offspring of:
Affected fathers
Affected mothers

Cell, Vol. 67, 1047–1058, December 20, 1991, Copyright © 1991 by Cell Press

Variation of the CGG Repeat at the Fragile X Site Results in Genetic Instability: Resolution of the Sherman Paradox

Ying-Hui Fu,* Derek P. A. Kuhl,* Antonio Pizzuti,*
Maura Pieretti,* James S. Sutcliffe,†
Stephen Richards,* Annemieke J. M. H. Verkerk,‡
Jeanette J. A. Holden,§ Raymond G. Fenwick, Jr.,*
Stephen T. Warren,† Ben A. Oostra,‡
David L. Nelson,* and C. Thomas Caskey*
*Institute for Molecular Genetics
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Houston, Texas 77030
†Departments of Biochemistry and Pediatrics
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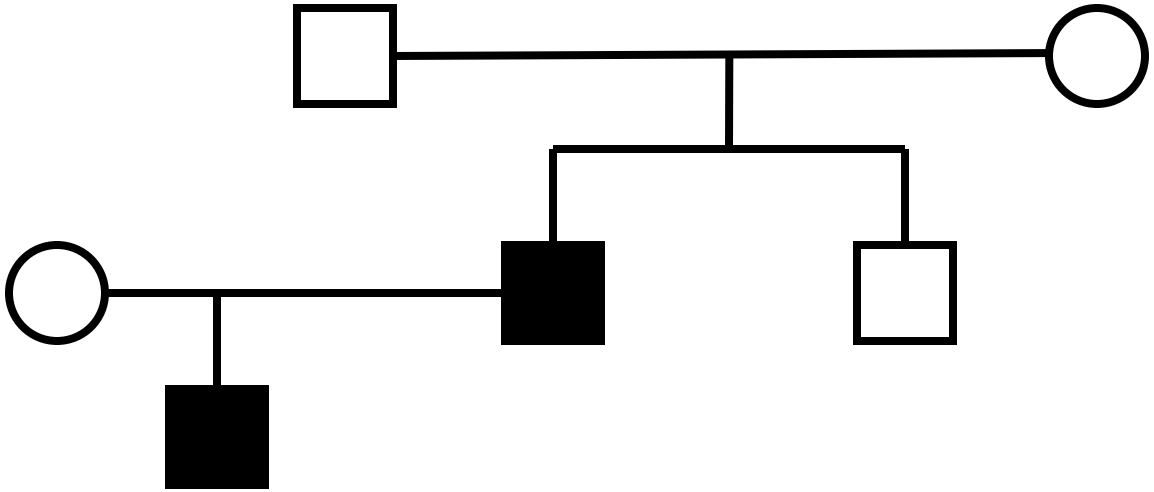
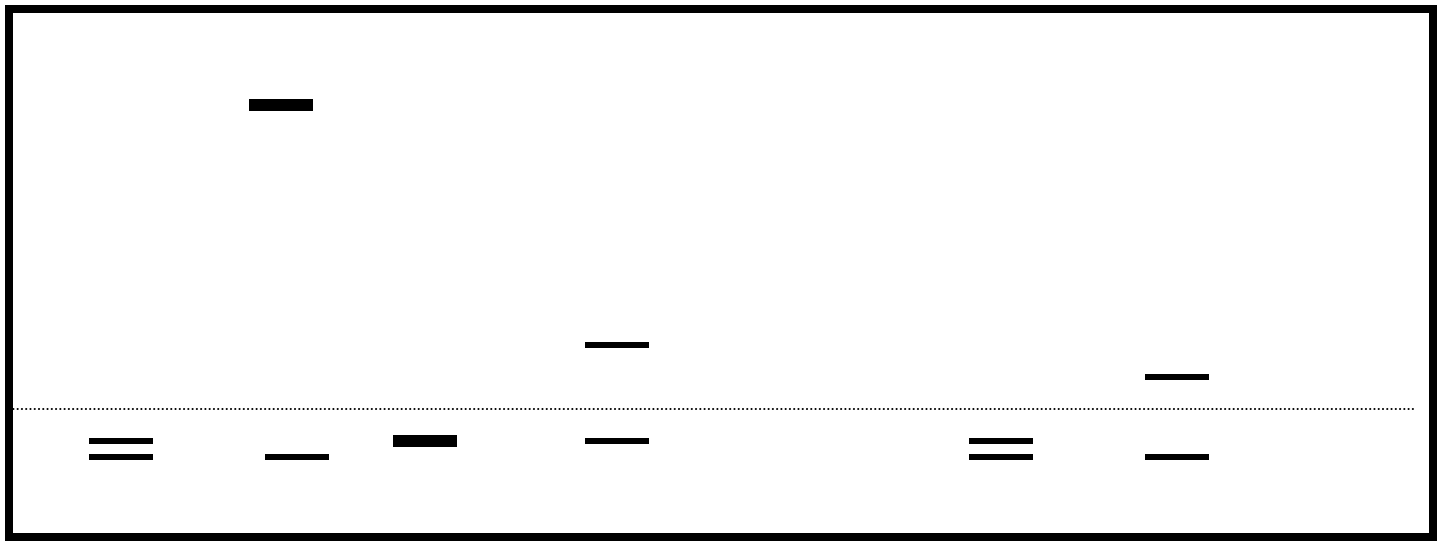
‡Department of Cell Biology
Erasmus University
Rotterdam
The Netherlands
§Department of Cytogenetics
Ongwanada Resource Center, Queens University
Kingston, Ontario, Canada K7L 3N6

The Molecular Basis of Anticipation

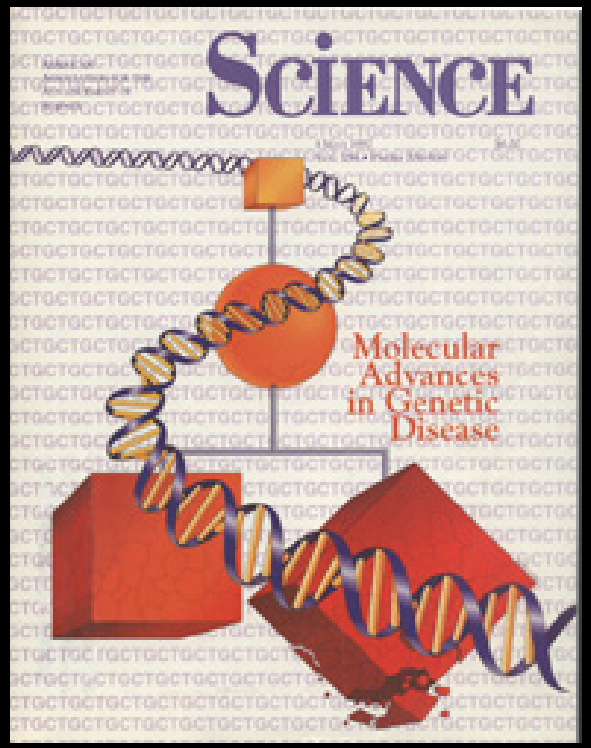




Myotonic dystrophy

The search for the DM1 gene

- Linked to ‘secretor’
- ‘Secretor’ mapped to chromosome 19
- Fine mapping of the DM1 locus
- Search for the DM1 gene
- A novel gene predicted to be a protein kinase



An Unstable Triplet Repeat in a Gene Related to Myotonic Muscular Dystrophy

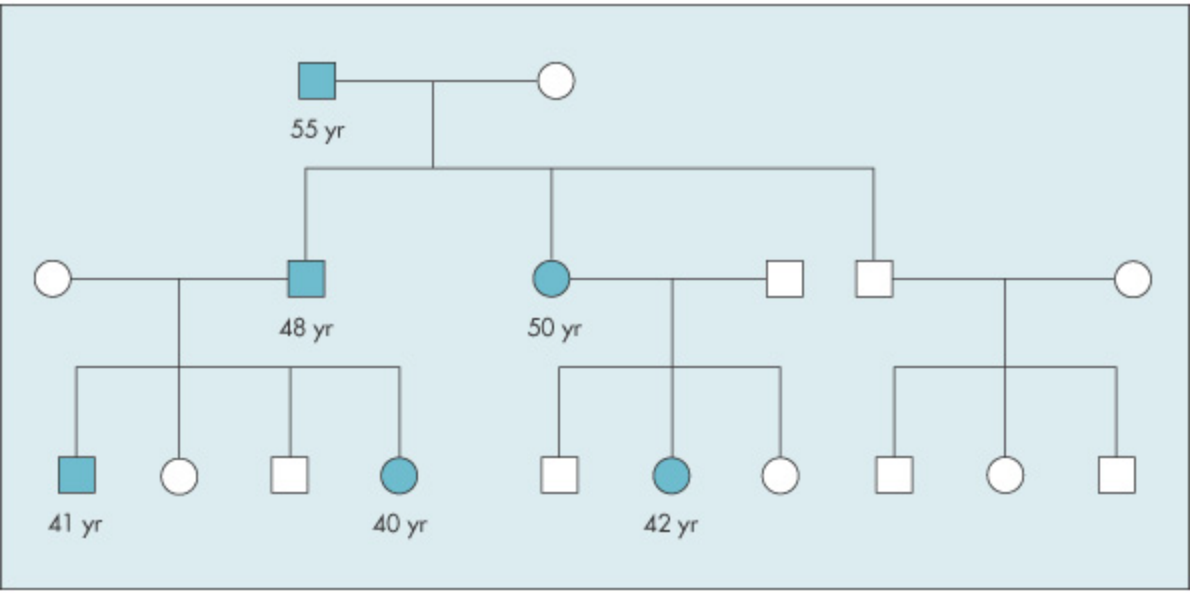
Y.-H. Fu, A. Pizzuti, R. G. Fenwick, Jr., J. King, S. Rajnarayan, P. W. Dunne, J. Dubel, G. A. Nasser, T. Ashizawa, P. de Jong, B. Wieringa, R. Korneluk, M. B. Perryman, H. F. Epstein, C. Thomas Caskey*†

Synthetic oligonucleotides containing GC-rich triplet sequences were used in a scanning strategy to identify unstable genetic sequences at the myotonic dystrophy (DM) locus. A highly polymorphic GCT repeat was identified and found to be unstable, with an increased number of repeats occurring in DM patients. In the case of severe congenital DM, the paternal triplet allele was inherited unaltered while the maternal, DM-associated allele was unstable. These studies suggest that the mutational mechanism leading to DM is triplet amplification, similar to that occurring in the fragile X syndrome. The triplet repeat sequence is within a gene (to be referred to as myotonin–protein kinase), which has a sequence similar to protein kinases.

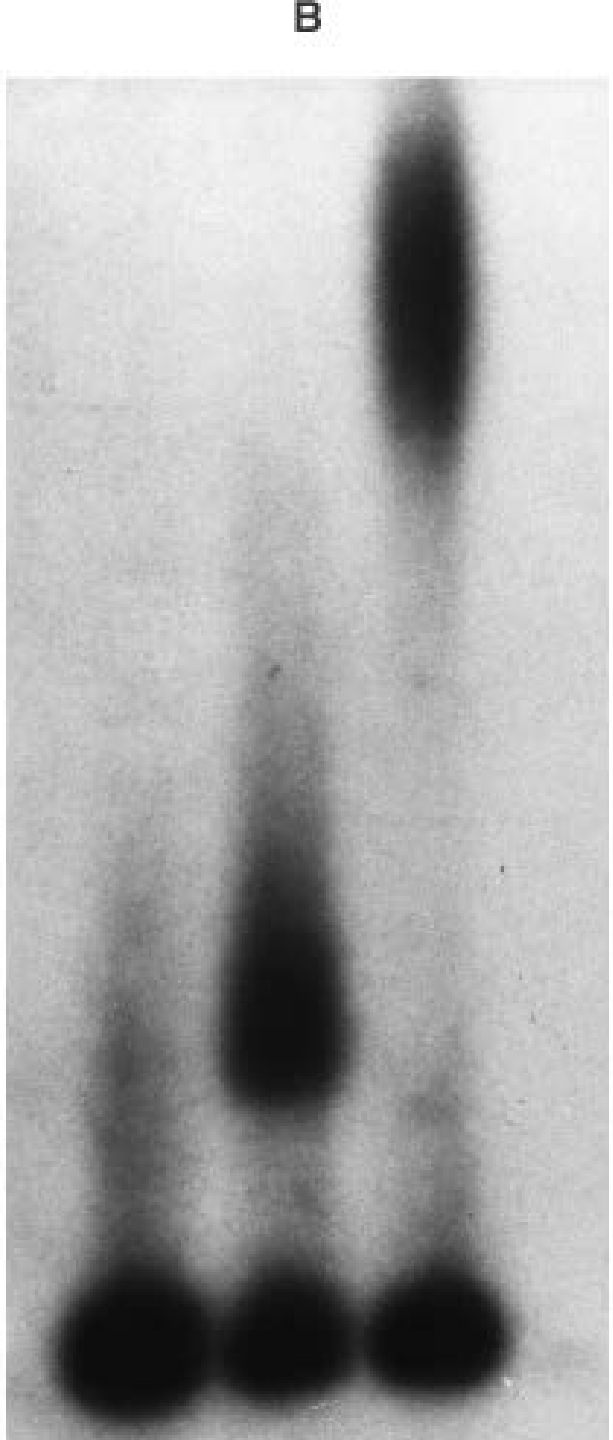
Science, 1992; 255:1256

Anticipation and Unstable repeats

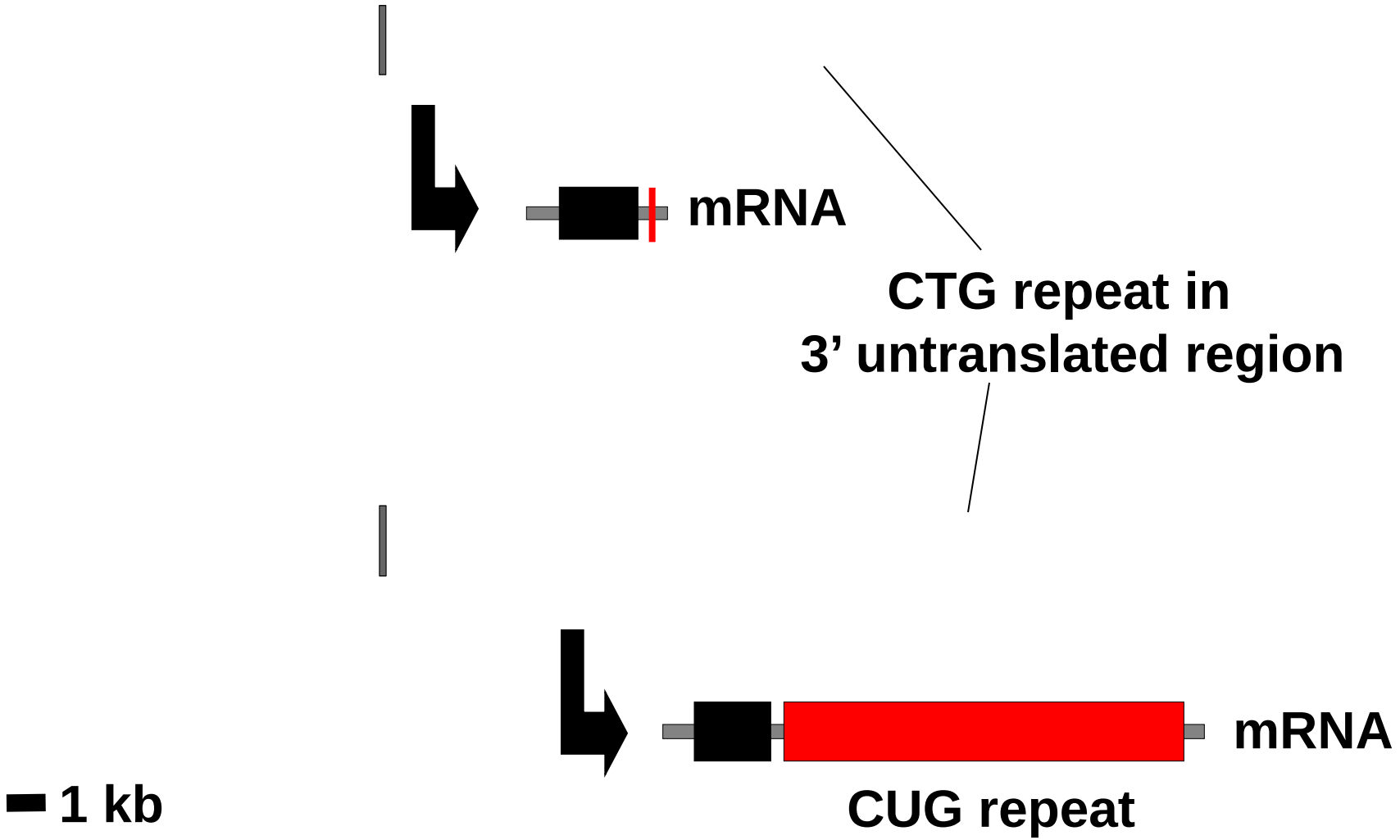
A



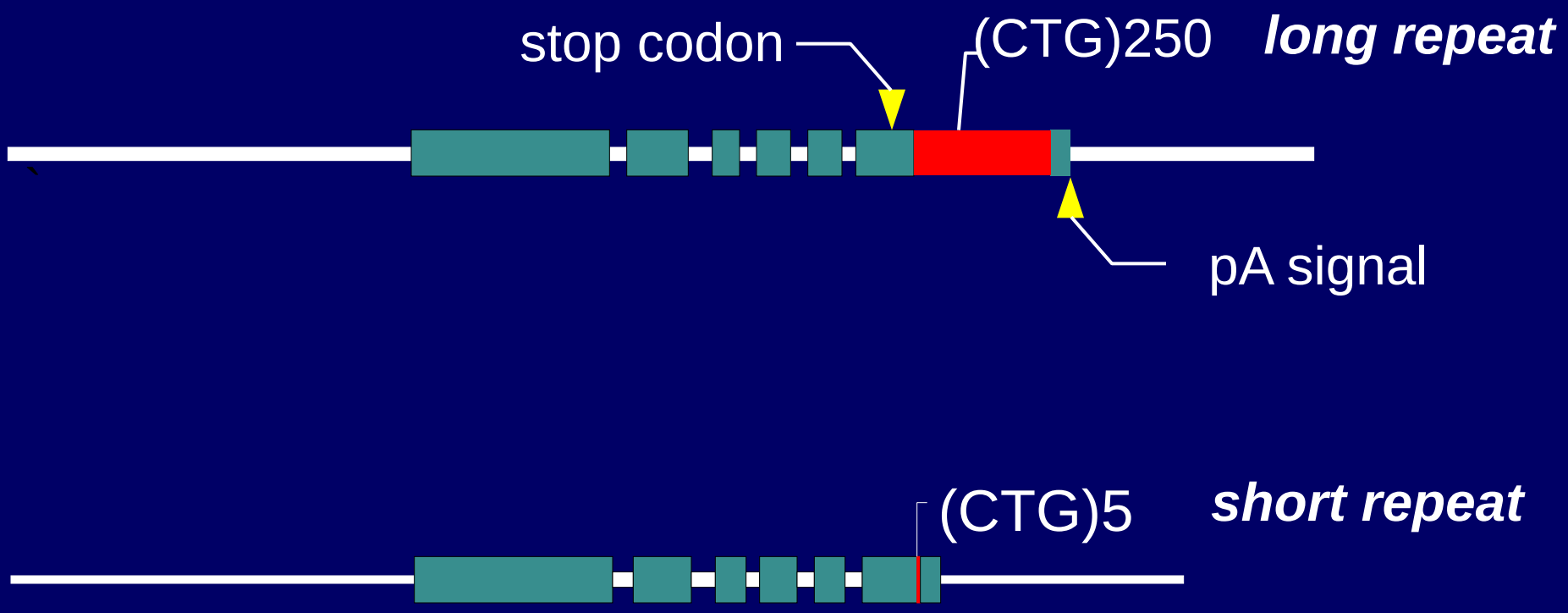
B



How does a 3’ UTR mutation cause disease?

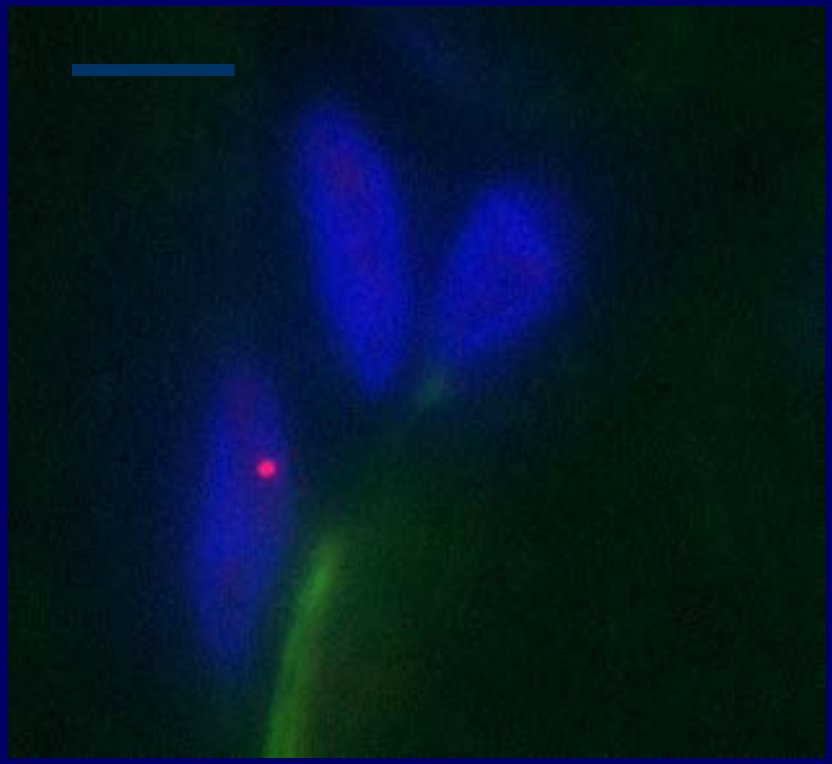


human skeletal actin (*HSA*) gene

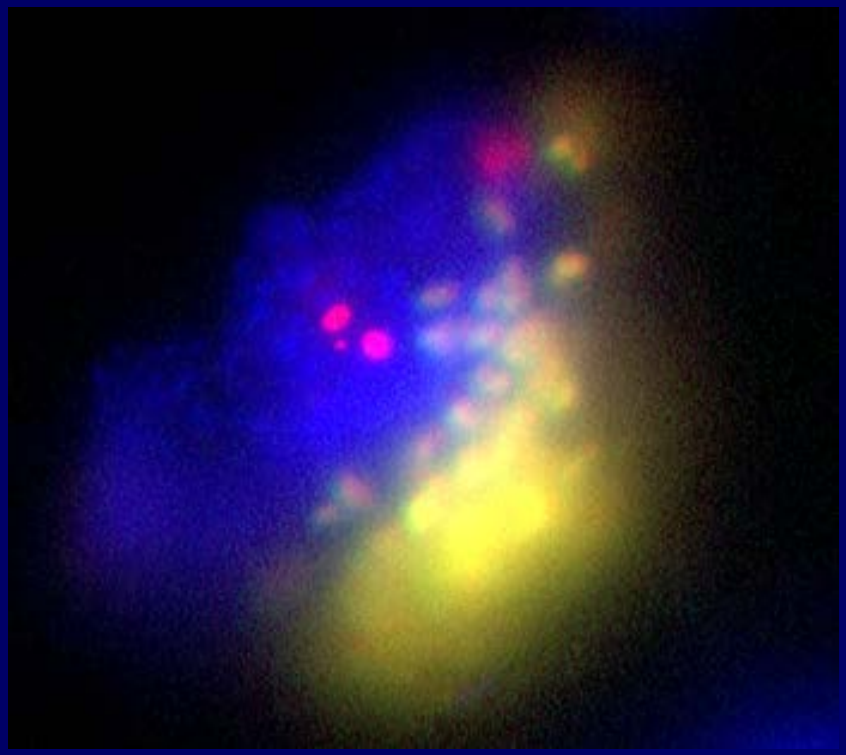


Transgene	# of lines of Tg mice	# lines with myotonia & myopathy
Short repeat	5	0
Long repeat	7	6

RNA expanded repeat forms inclusions in the nucleus

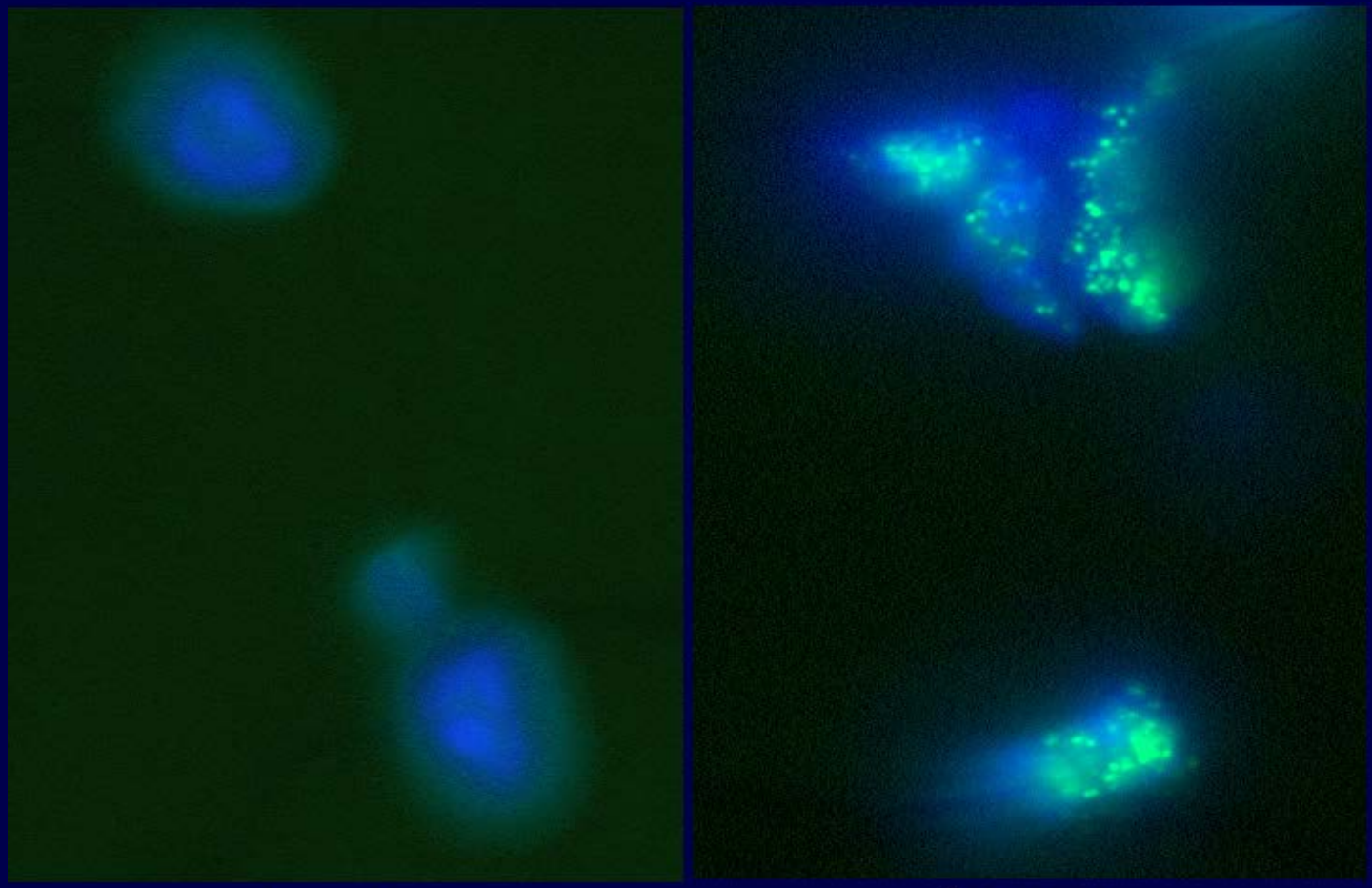


muscle cell



cortical neuron

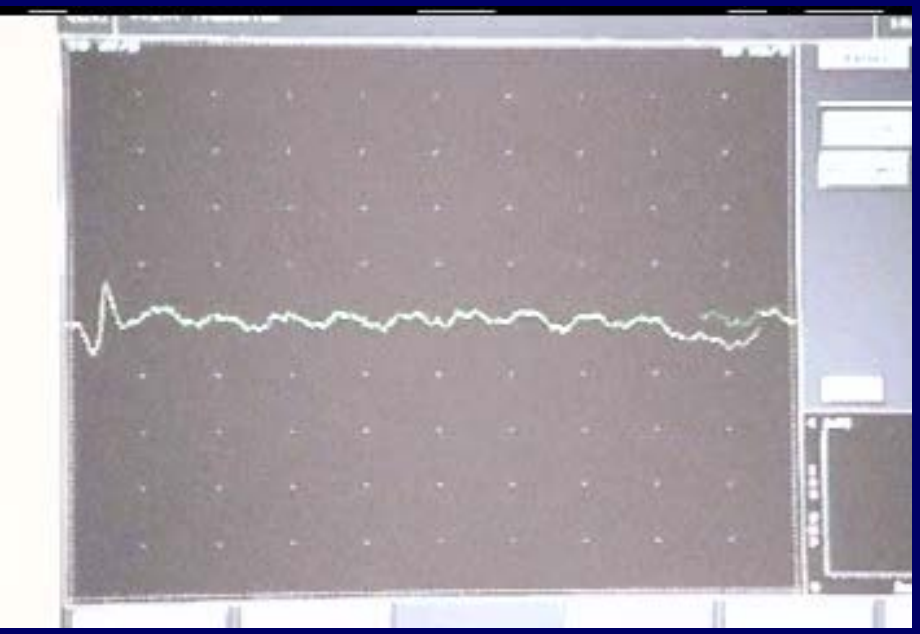
Charles Thornton, U Rochester



short-repeat

long-repeat

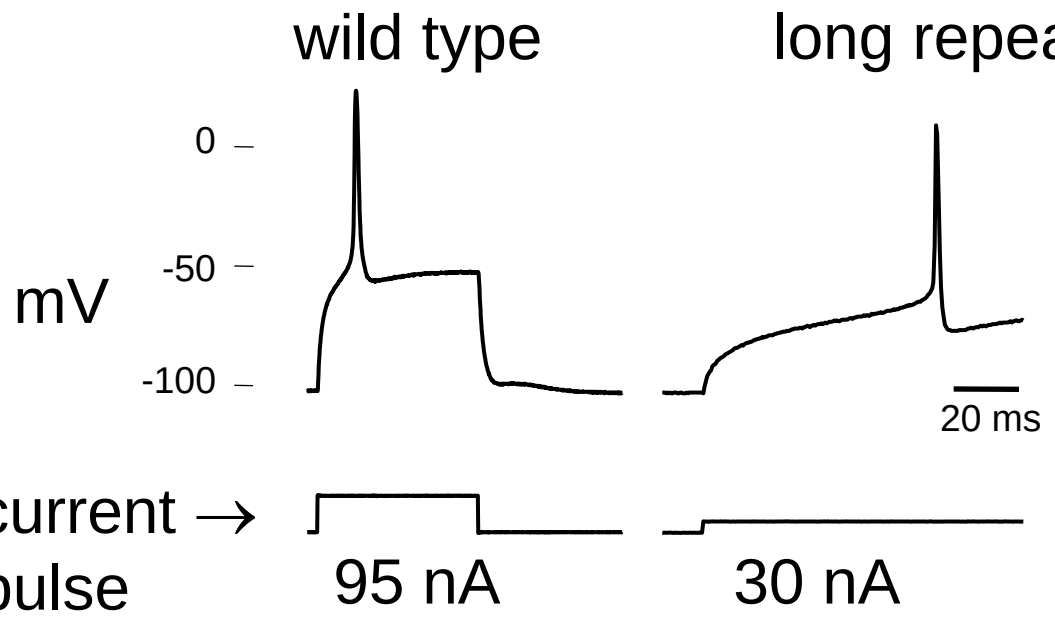
Charles Thornton, U Rochester



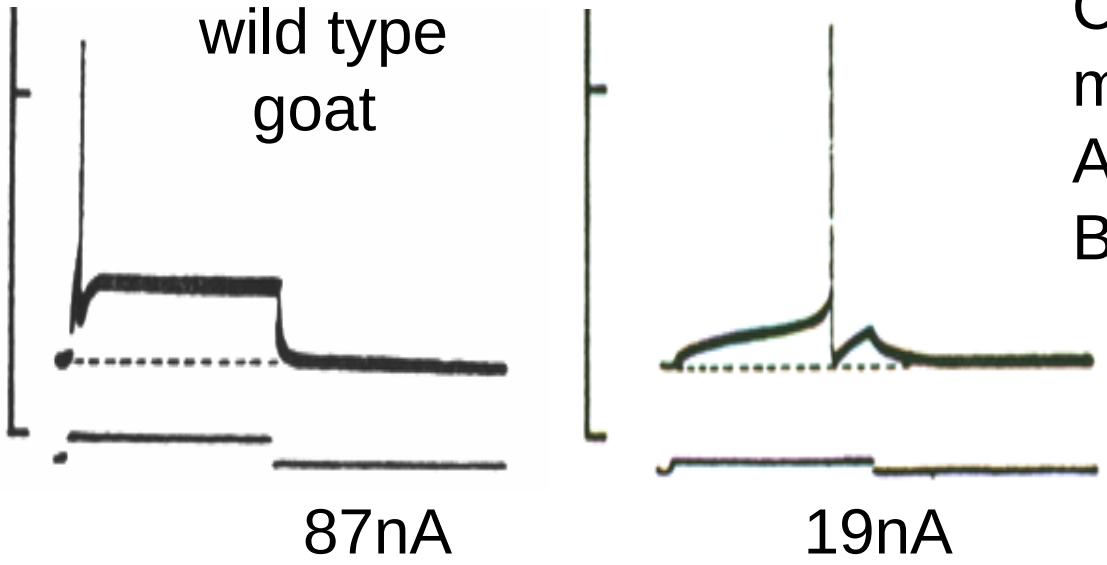
***HSA*^{SR} mice**

***HSA*^{LR} mice**

Charles Thornton, U Rochester

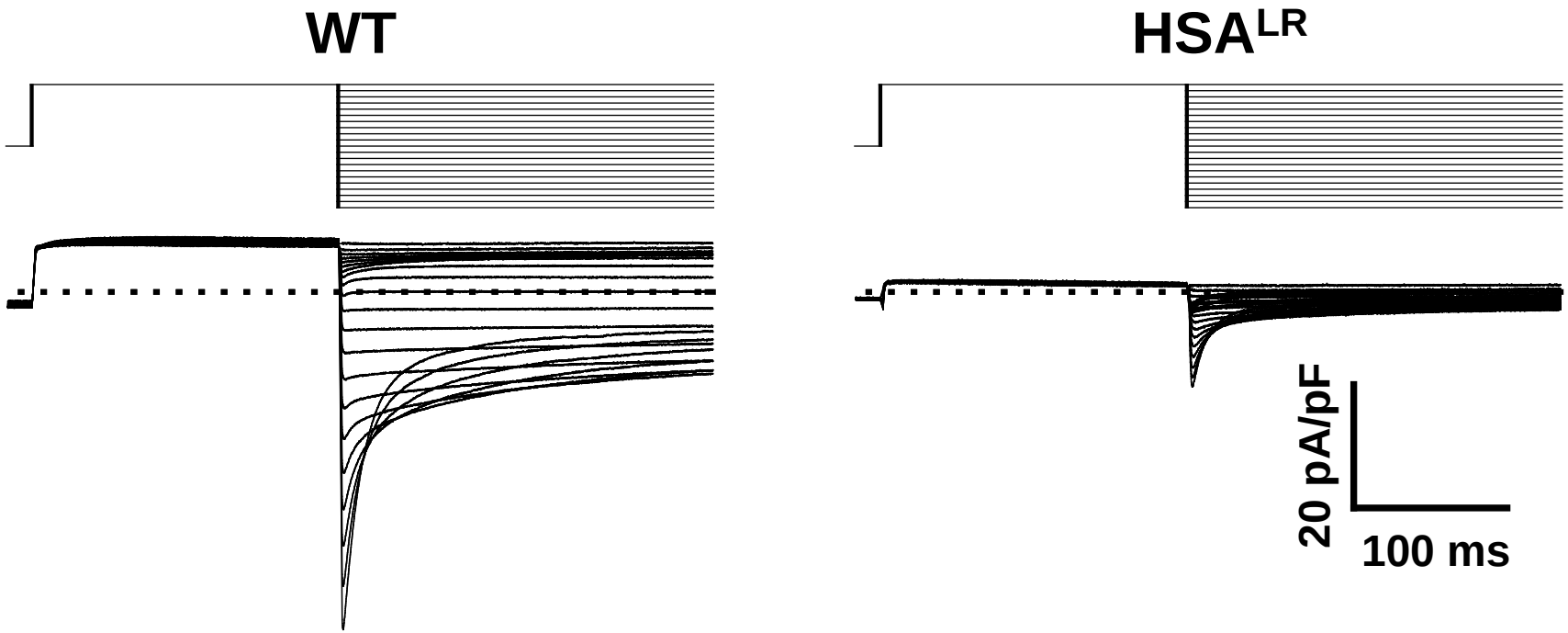


Takahashi & Cannon

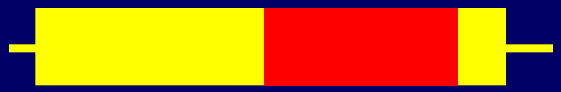


ClC1 -/- myotonic goat
Adrian & Bryant 1974

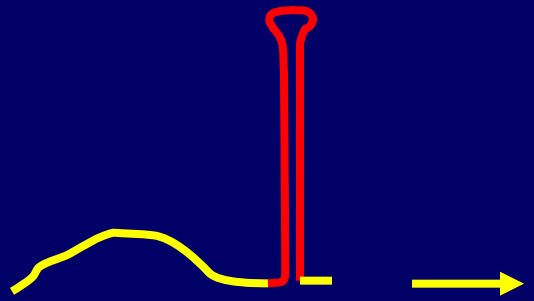
chloride channel 1 (ClC-1)
whole-cell patch clamp
flexor digitorum brevis fibers



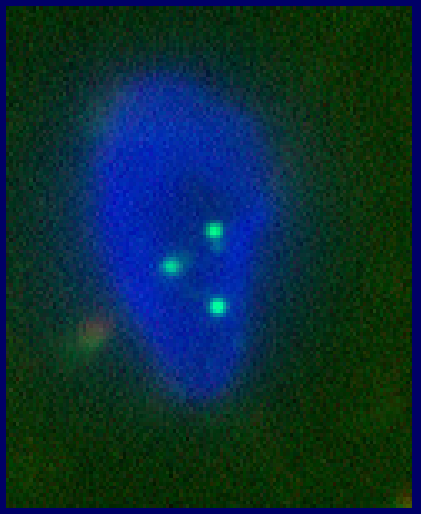
Bob Dirksen
John Lueck



expanded repeat
(DNA)



expanded repeat
(RNA)



nuclear RNA foci

myotonia

Split Genes

protein coding region (exons)

interruptions (introns)

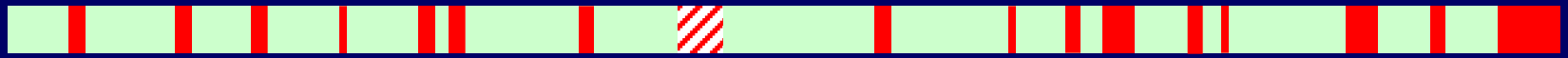
Gene

⇩ Transcription

Primary RNA (pre-mRNA)

⇩ RNA Splicing

mRNA



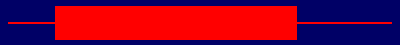
gene

transcription

primary transcript (pre-mRNA)

alternative splicing

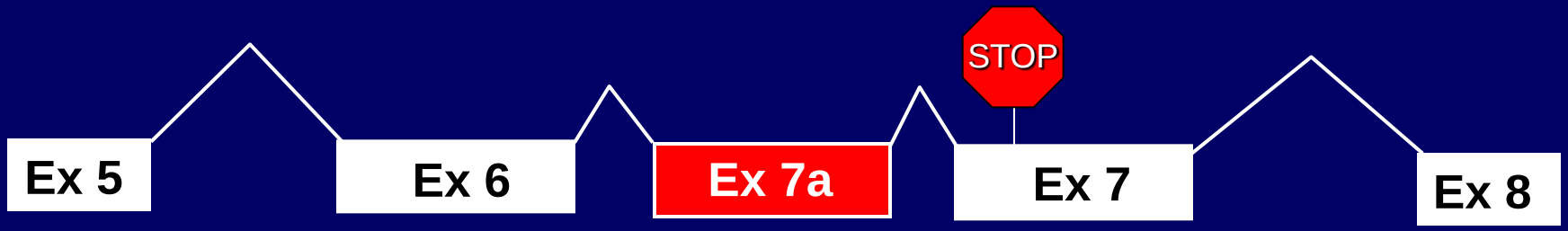
mRNA



isoform A

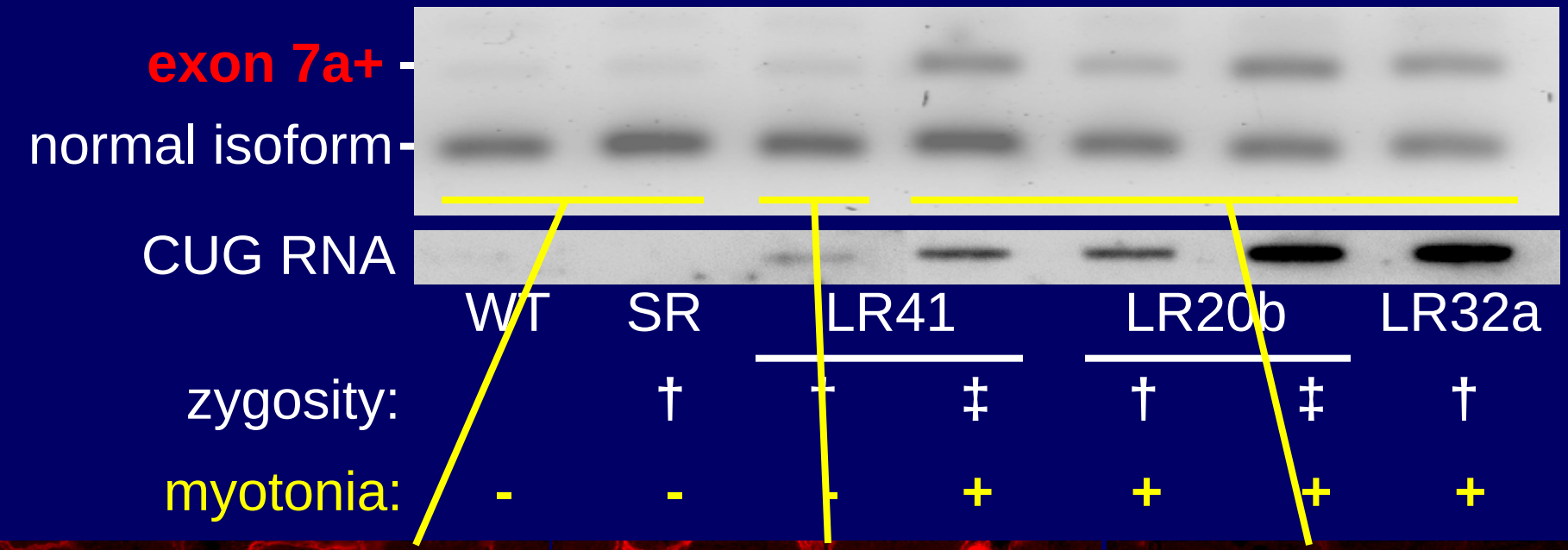
isoform B

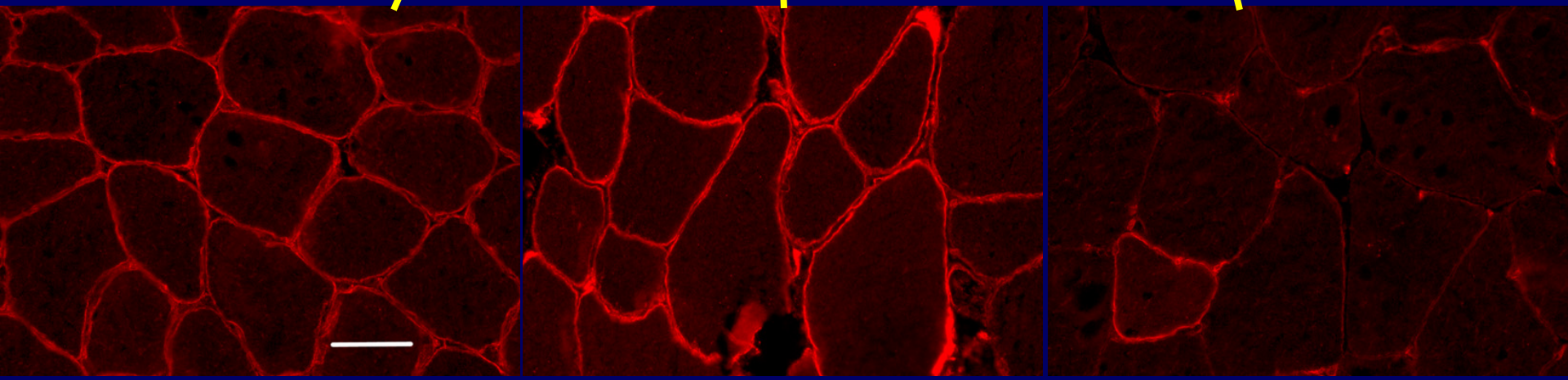
Alternative splicing of ClC-1 chloride channel in long repeat transgenic mice



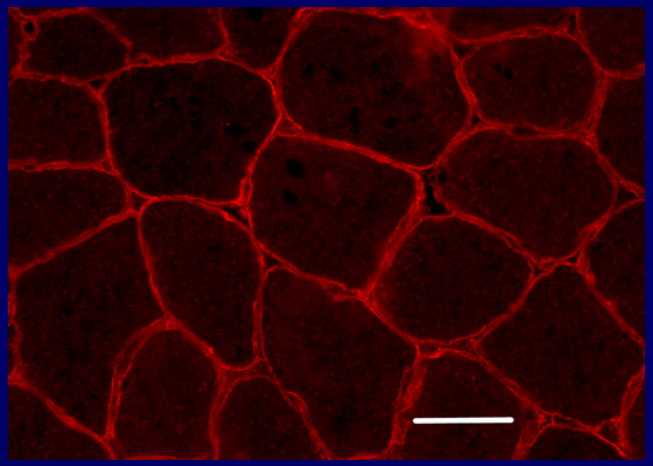
Charles Thornton, U Rochester

ClC-1 splicing (exons 5 to 8)

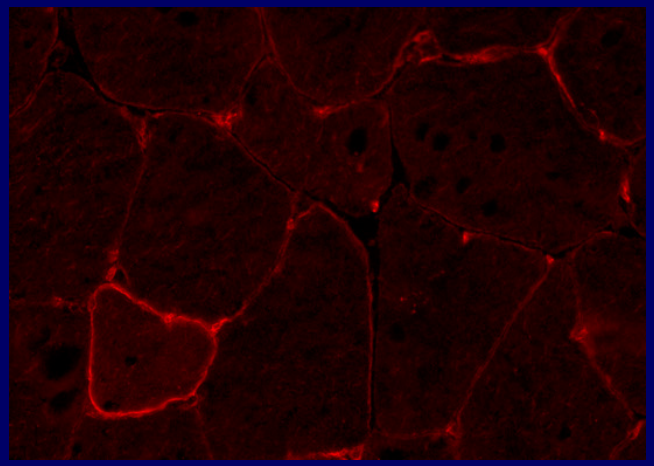




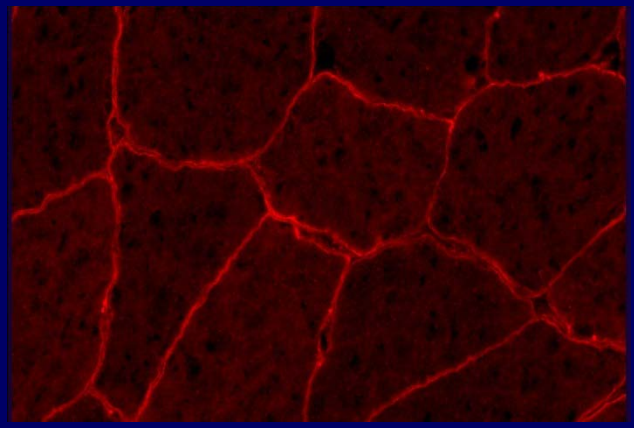
ClC1 chloride channel



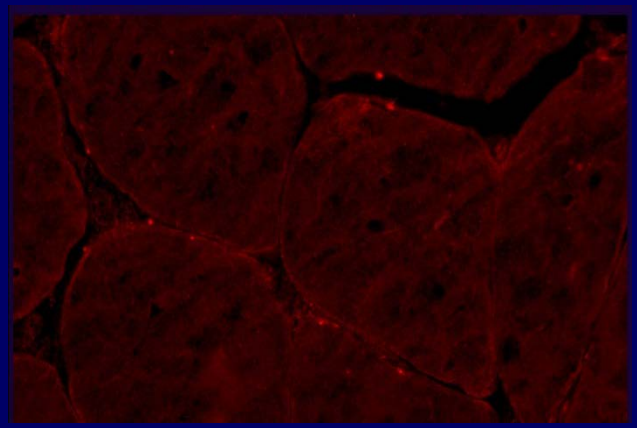
wild type mouse



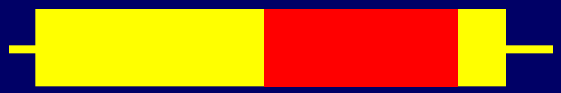
HSA^{LR} Tg mouse



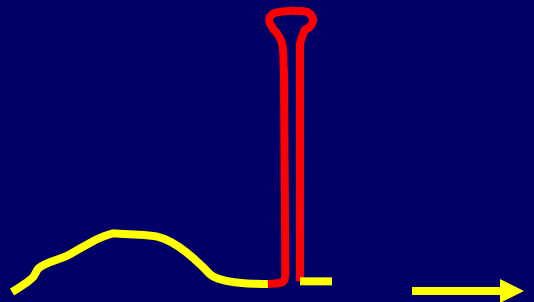
normal human muscle



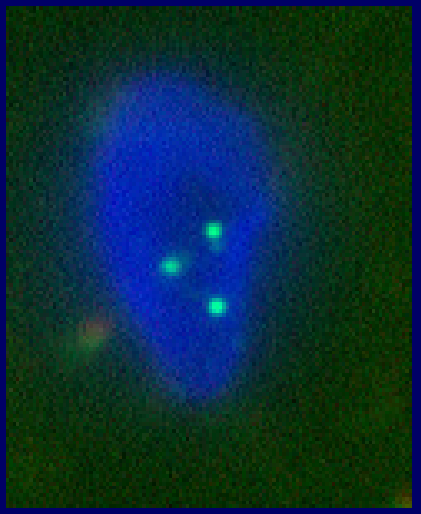
myotonic dystrophy



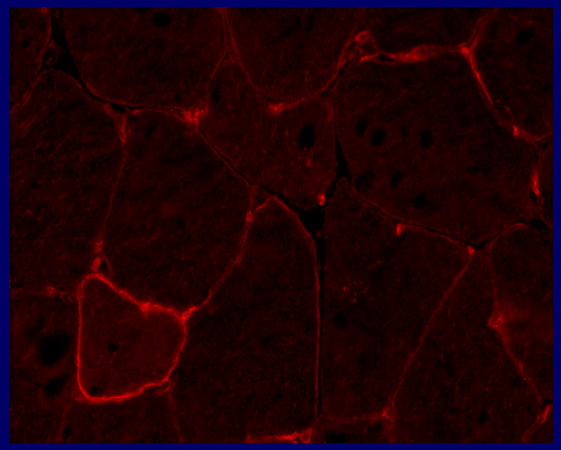
expanded repeat
(DNA)



expanded repeat
(RNA)



nuclear
RNA foci

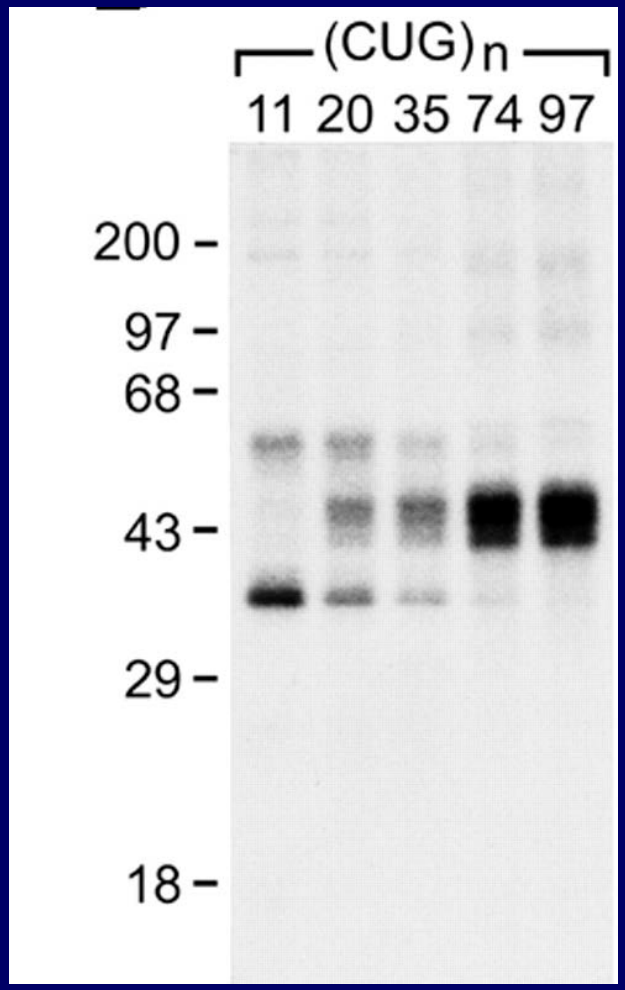


myotonia

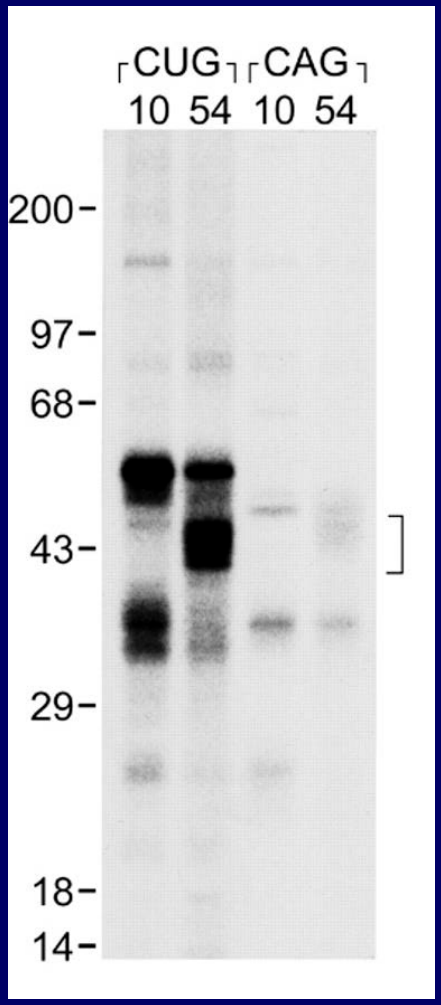
trans-interference
with regulation of
alternative splicing

loss of chloride channel

Recruitment of human muscleblind proteins to $(CUG)_n$ expansions associated with DM1

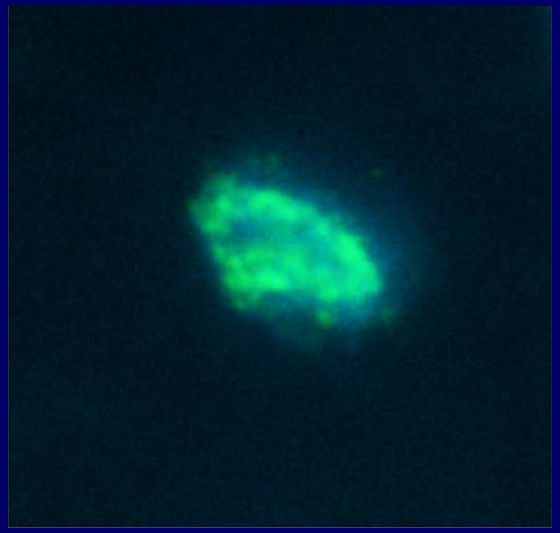


Muscleblind (MBNL1)

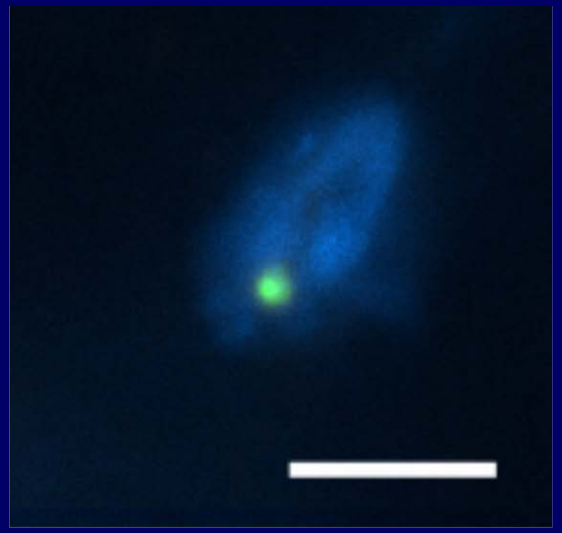


JW Miller et al, 2000

Distribution of Muscleblind 1 in the muscle nucleus

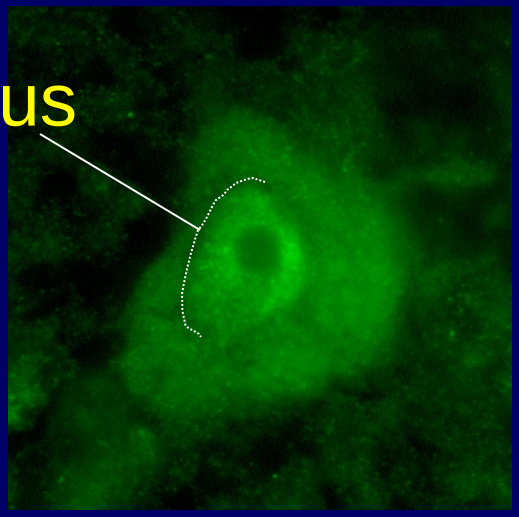


Normal



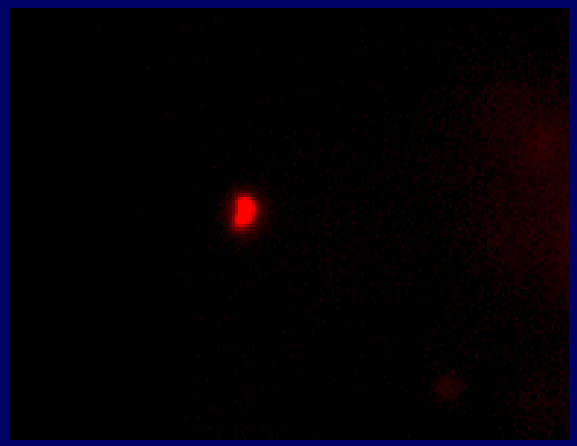
DM1

nucleus

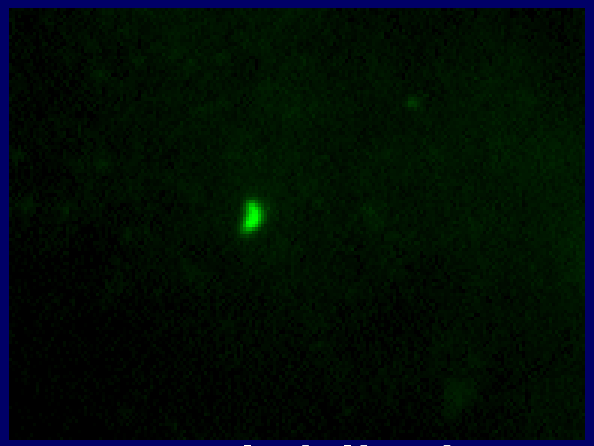


non-disease control

Distribution of muscleblind 1 protein in cortical neurons



triplet repeat RNA



muscleblind 1

merge

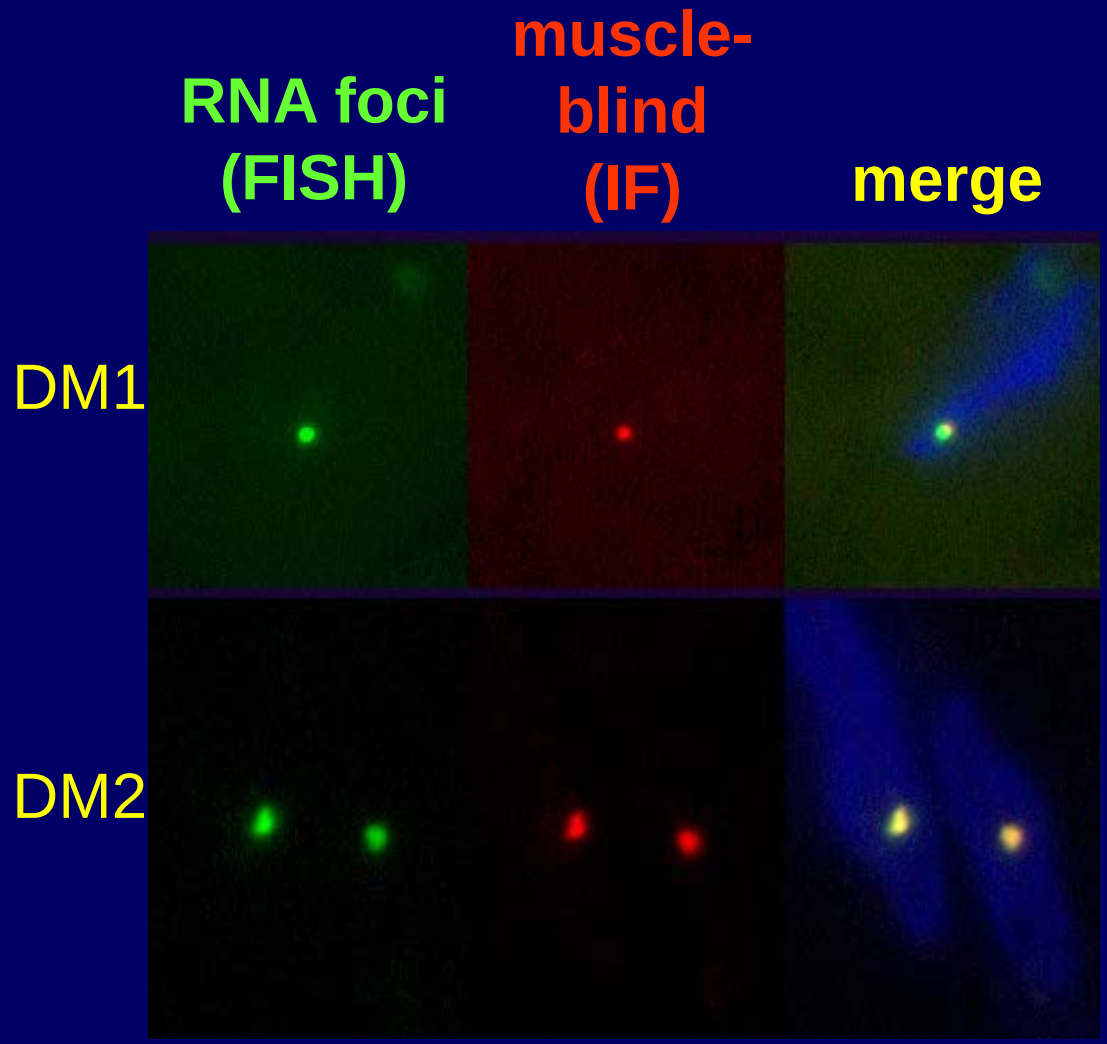
DM1

Myotonic Dystrophy Type 2 Caused by a CCTG Expansion in Intron 1 of *ZNF9*

Christina L. Liquori,[1,2] Kenneth Ricker,[4] Melinda L. Moseley,[1,2] Jennifer F. Jacobsen,[1,2] Wolfram Kress,[5] Susan L. Naylor,[6] John W. Day,[1,3]* Laura P. W. Ranum[1,2]*

Myotonic dystrophy (DM), the most common form of muscular dystrophy in adults, can be caused by a mutation on either chromosome 19q13 (DM1) or 3q21 (DM2/PROMM). DM1 is caused by a CTG expansion in the 3′ untranslated region of the dystrophia myotonica–protein kinase gene (*DMPK*). Several mechanisms have been invoked to explain how this mutation, which does not alter the protein-coding portion of a gene, causes the specific constellation of clinical features characteristic of DM. We now report that DM2 is caused by a CCTG expansion (mean ~5000 repeats) located in intron 1 of the zinc finger protein 9 (*ZNF9*) gene. Parallels between these mutations indicate that microsatellite expansions in RNA can be pathogenic and cause the multisystemic features of DM1 and DM2.

Science 2001; 293: 864



muscle nuclei

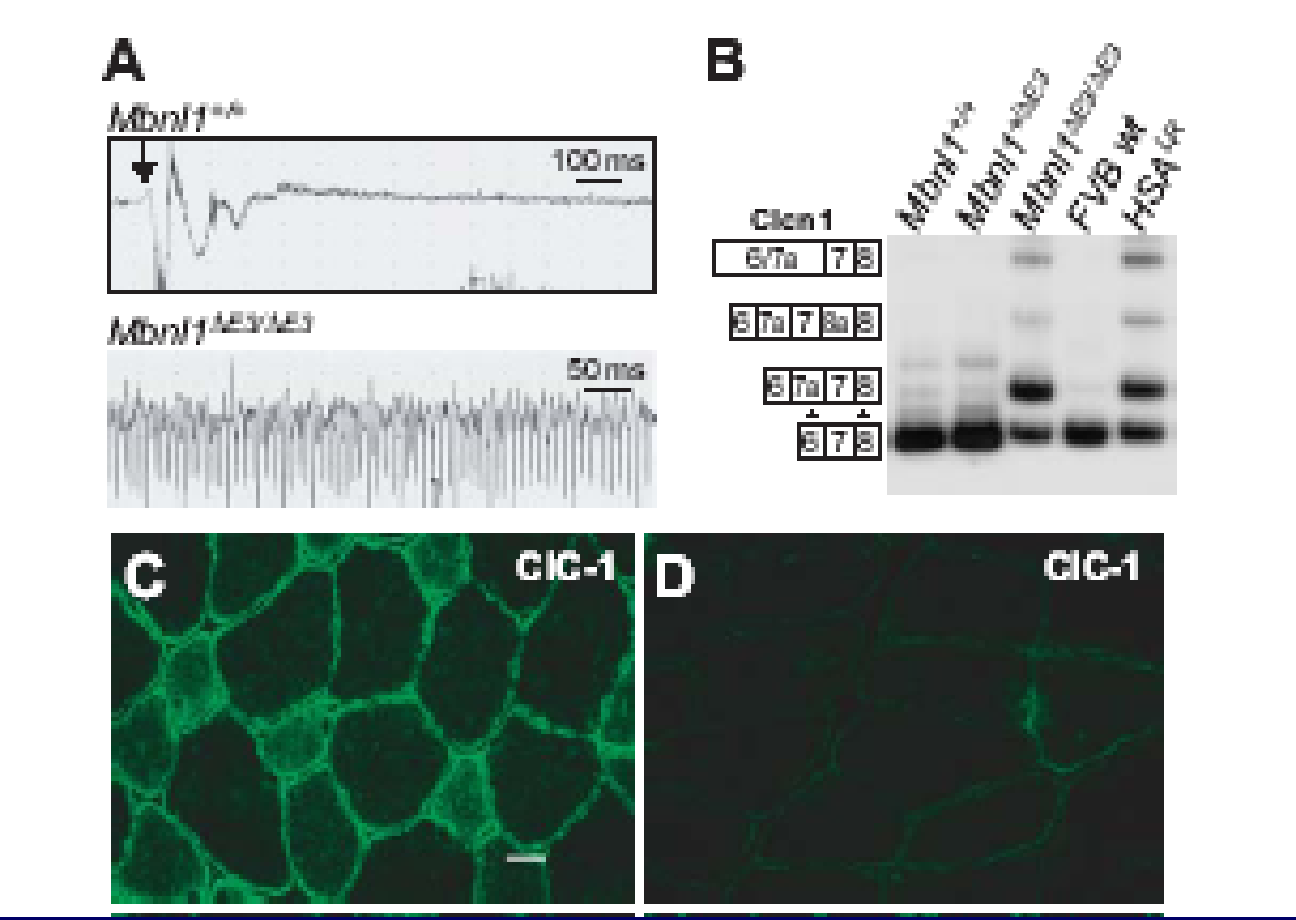
A Muscleblind Knockout Model for Myotonic Dystrophy

Rahul N. Kanadia,[1] Karen A. Johnstone,[1] Ami Mankodi,[3] Codrin Lungu,[3] Charles A. Thornton,[3] Douglas Esson,[2] Adrian M. Timmers,[2] William W. Hauswirth,[2] Maurice S. Swanson[1]*

The neuromuscular disease myotonic dystrophy (DM) is caused by microsatellite repeat expansions at two different genomic loci. Mutant DM transcripts are retained in the nucleus together with the muscleblind (Mbnl) proteins, and these abnormal RNAs somehow interfere with pre-mRNA splicing regulation. Here, we show that disruption of the mouse *Mbnl1* gene leads to muscle, eye, and RNA splicing abnormalities that are characteristic of DM disease. Our results support the hypothesis that manifestations of DM can result from sequestration of specific RNA binding proteins by a repetitive element expansion in a mutant RNA.

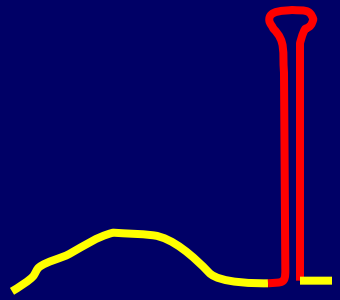
Science 2003;302:1978

Isoform specific knockout of *Mbnl1*

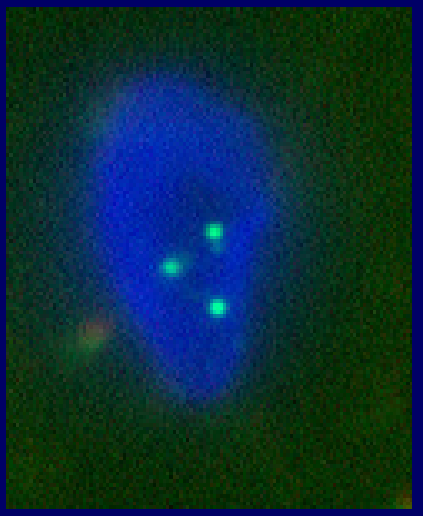


wild-type *Mbnl1*$^{\Delta ex3/\Delta ex3}$

$(CTG)_n$ or $(CCTG)_n$
(gene)



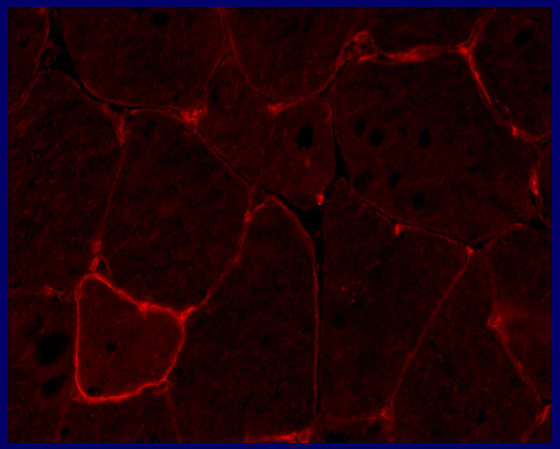
$(CUG)_n$ or $(CCUG)_n$
(RNA)



RNA inclusions

sequestration of muscleblind protein

abnormal splicing of pre-mRNA



loss of ClC-1 protein

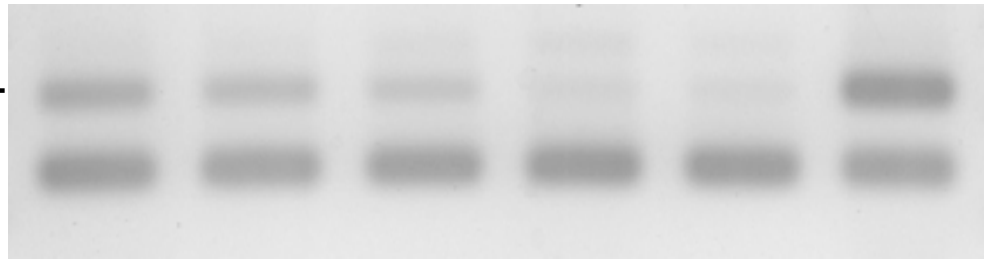
myotonia

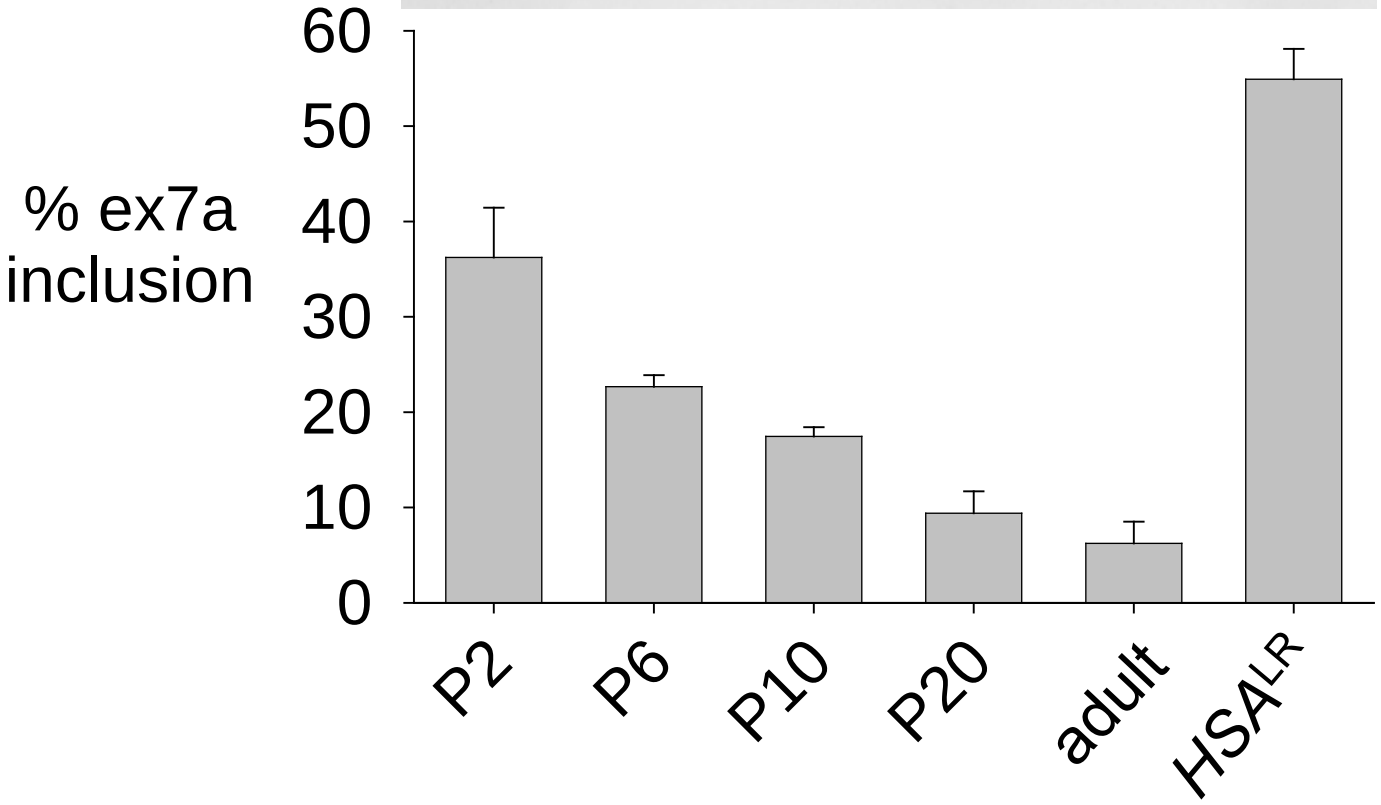
Postnatal regulation of ClC-1 splicing in WT mice

~~Channel~~

ex7a+

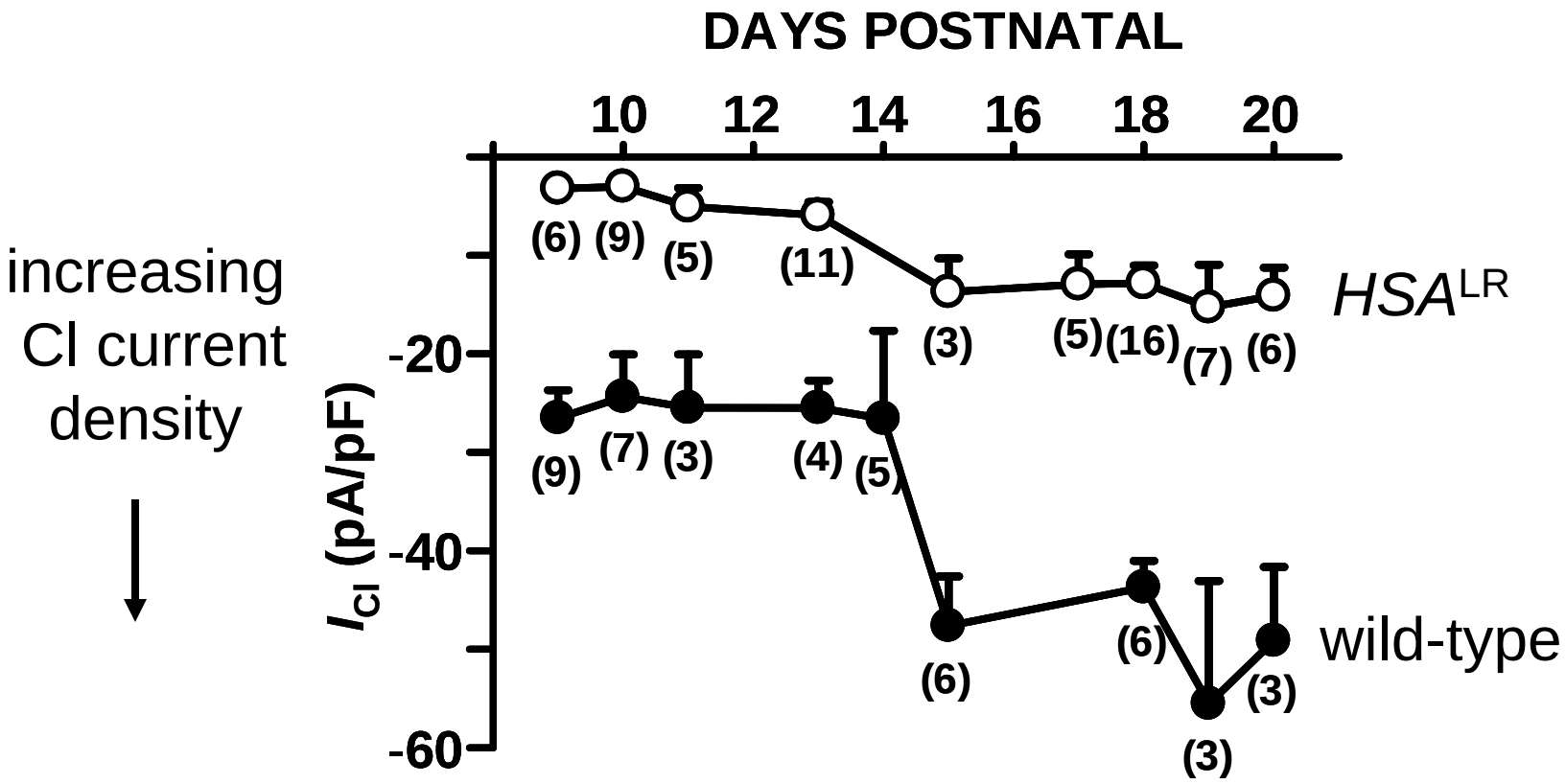
ex7a-





P=postnatal day

Clc1 current density in FDB fibers



Bob Dirksen
John Lueck

Summary of DM splicing story

Myotonia in DM results mainly from Cl channelopathy

↳ Cl channelopathy results from a splicing defect

↳ Splicing defect:

1. can be explained by loss of function for a single splicing factor, MBNL1
2. results from sequestration of MBNL1 in nuclear foci of RNA
3. similar in DM1, DM2, and mouse models
4. phenotypes in DM result partly from expression in mature muscle of splice isoforms that normally occur in immature muscle
5. compromise a developmental program of alternative splicing

One view of Human Genetics