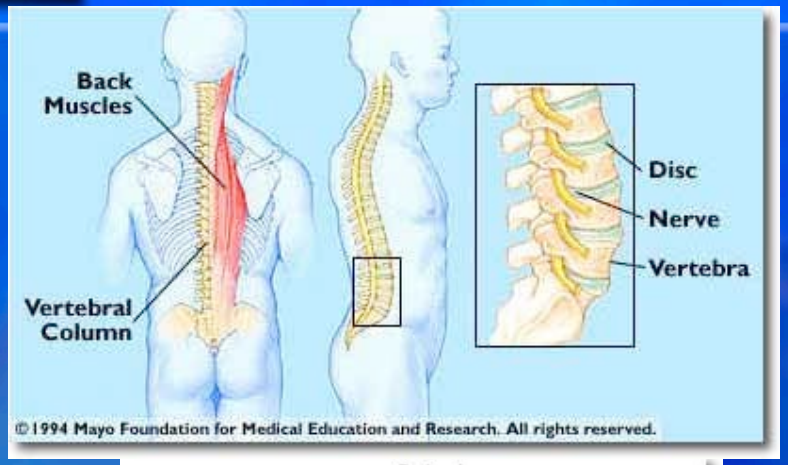
Kennedy's Disease: Mouse Models and Mechanistic Studies

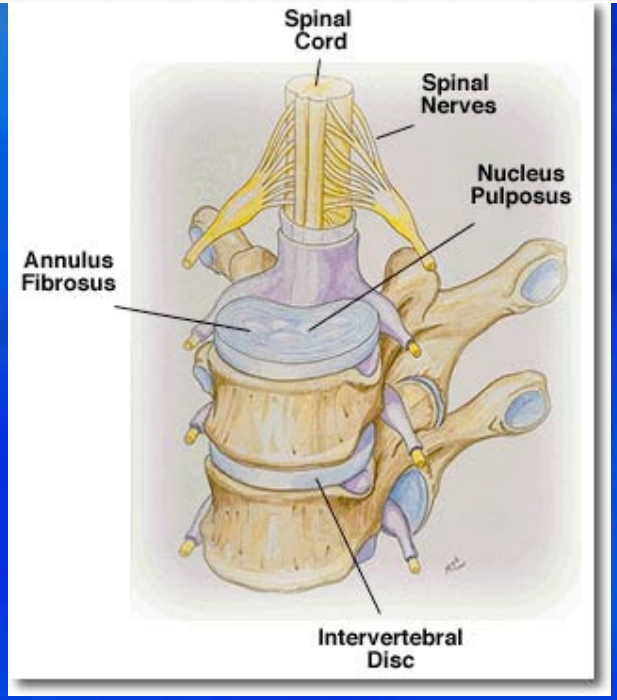
Outline

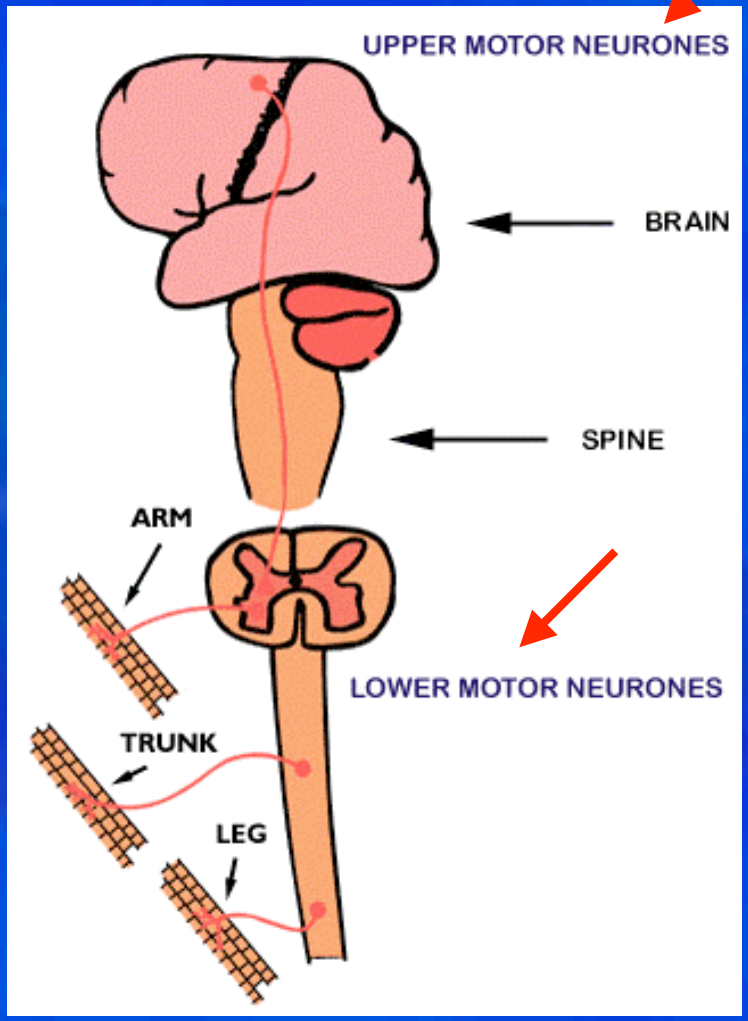
- Kennedy's Disease-Spinal Bulbar Muscular Atrophy
- Mouse Models of SBMA
- Transcriptional dysregulation-motor neuron loss
- Role of testosterone in SMBA
- Proteolysis and transcriptional dysregulation

Kennedy's Disease: What kind of disease is it?

Motor Neuron Disease





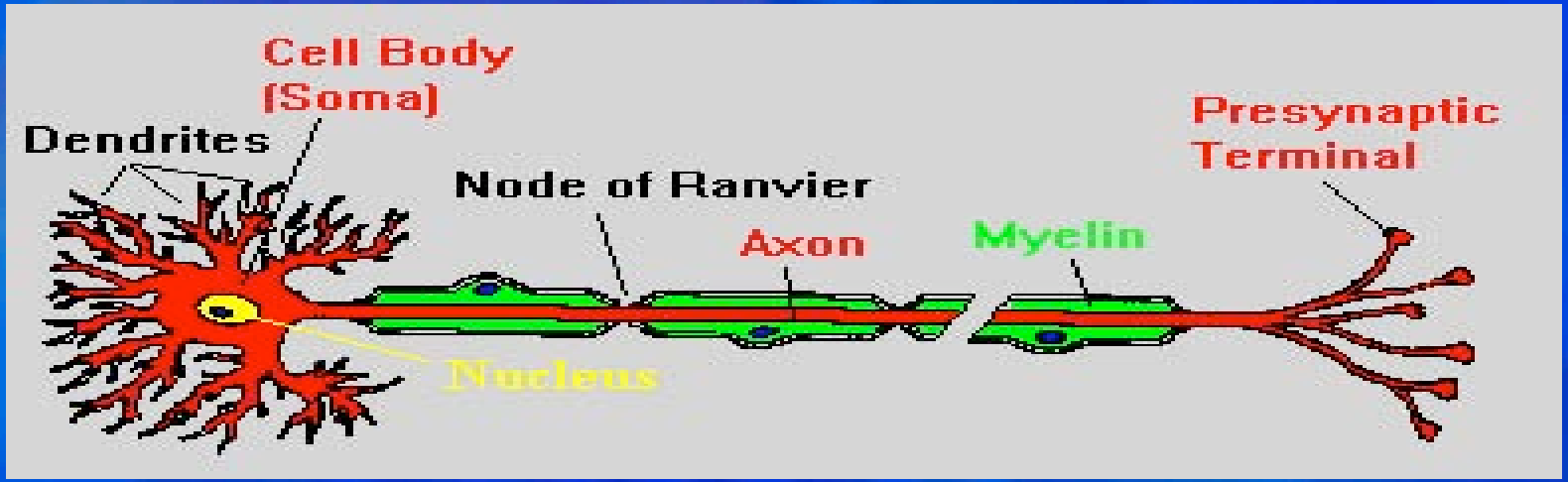


Motor Neuron Diseases

- Amyotrophic Lateral Sclerosis or Lou Gehrig's disease affects both the lower and upper motor neurons-Genetic and environmental factors
- Polio effects the lower motor neurons and is caused by the polio virus
- Spinal Muscular Atrophy (SMA) affects the lower motor neurons-one that minics some of the features of Lou Gehrig's disease is ***Kennedy's disease or X-linked Spinobulbar Muscular Atrophy (SBMA)***

- There are 1 in 50,000 or 5,500 people diagnosed with motor neurons disease each year in the USA

Movement occurs through the action of motor neurons



Anatomy of motor neuron is special: Very long axon 10,000 times the length of the cell body

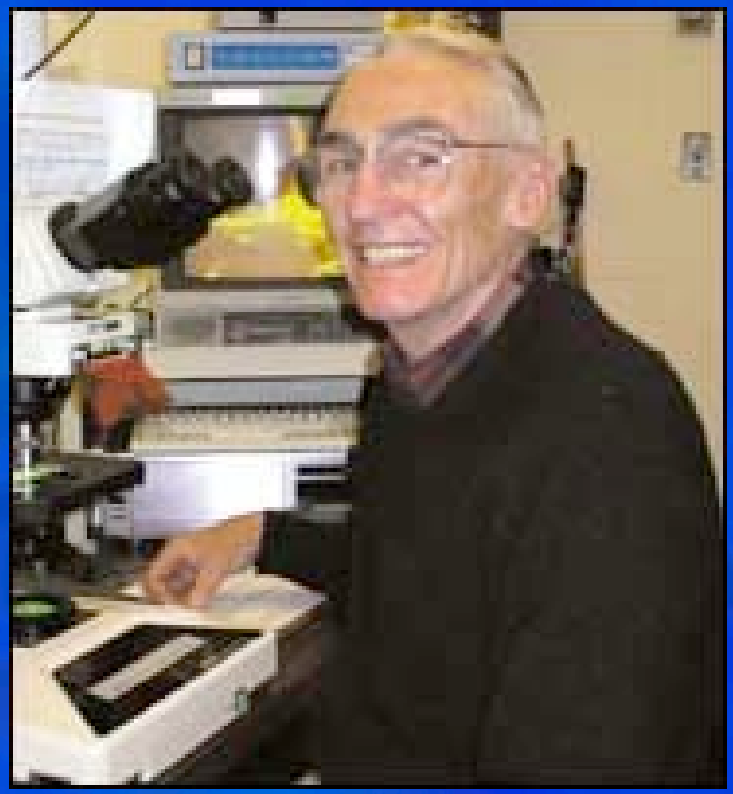
How was Kennedy's Disease Discovered?

- William R. Kennedy-neurologist at University of Minnesota

- 1964 George B. was referred to Kennedy-had a possible diagnosis of ALS

- ALS has upper and lower motor neuron loss-George had only lower motor neuron loss symptoms

- Progressive weakness and muscle twitches



Kennedy's Disease Symptoms

EFFECT OF MOTOR NEURON DEGENERATION

- Muscle weakness and wasting
- Swallowing difficulties-bulbar muscles/brain stem
- Speech dysfunction
- Shaky muscles
- Muscle twitches
- Absent reflexes

EFFECT OF MALE HORMONE DYSREGULATION

- Enlarged breast
- Low sperm count
- Shrunken testicles

What is the genetic mutation that causes Kennedy's Disease?

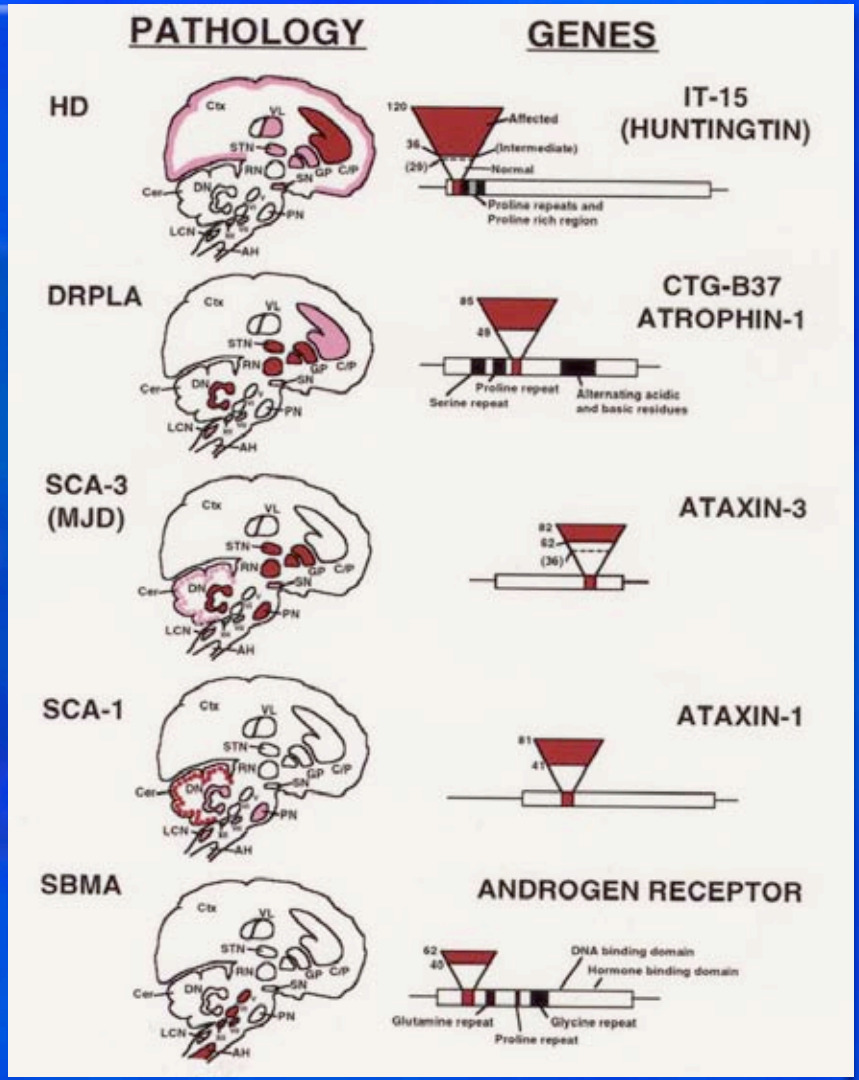
Kennedy's Disease

- SBMA is caused by a polyQ expansion (CAG expansion) in the N-terminus of androgen receptor.
- Expansion > 36-38 polyglutamine repeats causes SBMA
- Longer repeat length earlier age of onset of the disease.

Al La Spada and Kenneth Fischbeck, 1991

Polyglutamine Neurodegenerative Disorders

- At least eight are currently known (HD, DRPLA, SCA-1, 2, 3, 7, 17, and SBMA)
- DRPLA is most like HD
- Affected brain regions include cortex, basal ganglia, brainstem, cerebellum and spinal cord
- The genes share no homology except for the poly-Q repeats
- The androgen receptor is a DNA-binding transcription factor



Ross, Neuron 1995

Androgen Receptor Action

[1] steroid binds to receptor

[2] release heat shock proteins

[3] conformational change

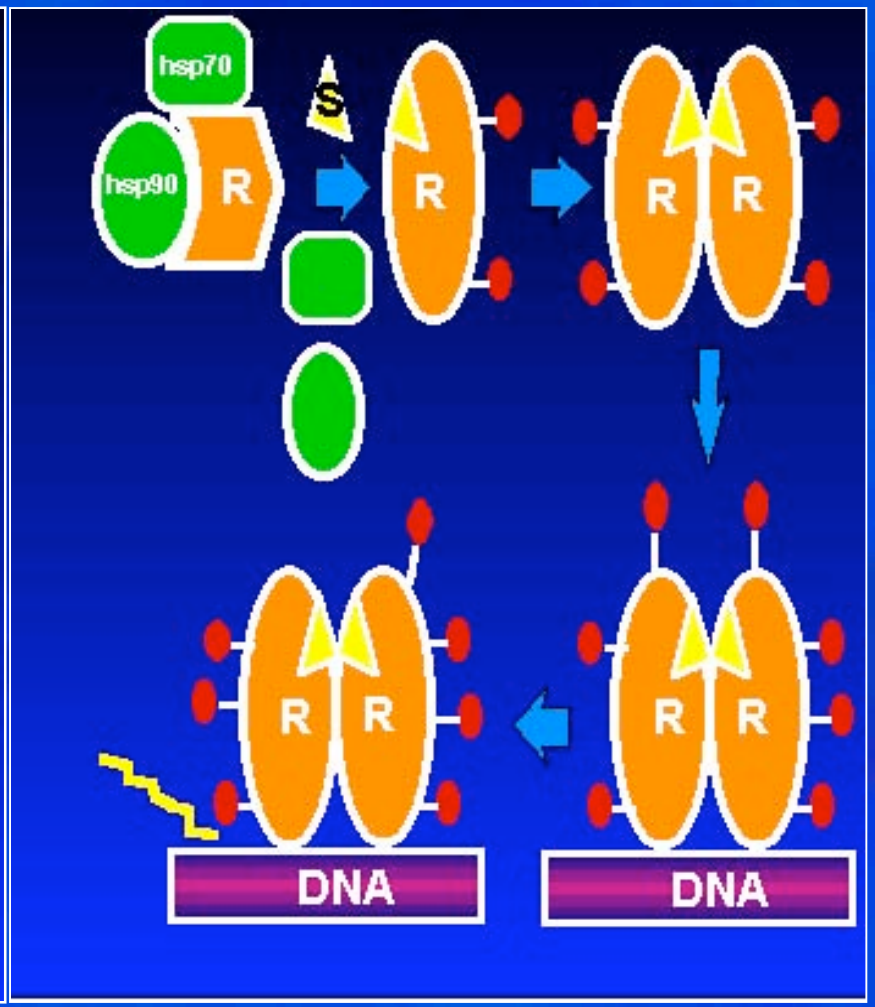
[4] phosphorylation

[5] dimerization

[6] binding to DNA

[7] further phosphorylation

[8] activation of transcription



Kennedy's Disease-Gain of Function

- Loss of function mutations in AR in patients causes feminization but not motor neuron loss.
- Expansion of polyglutamine repeat has no effect on hormone binding and only slightly reduces its ability to transactivate genes
- Partial loss of function-gynecomastia, predominantly *gain of function*

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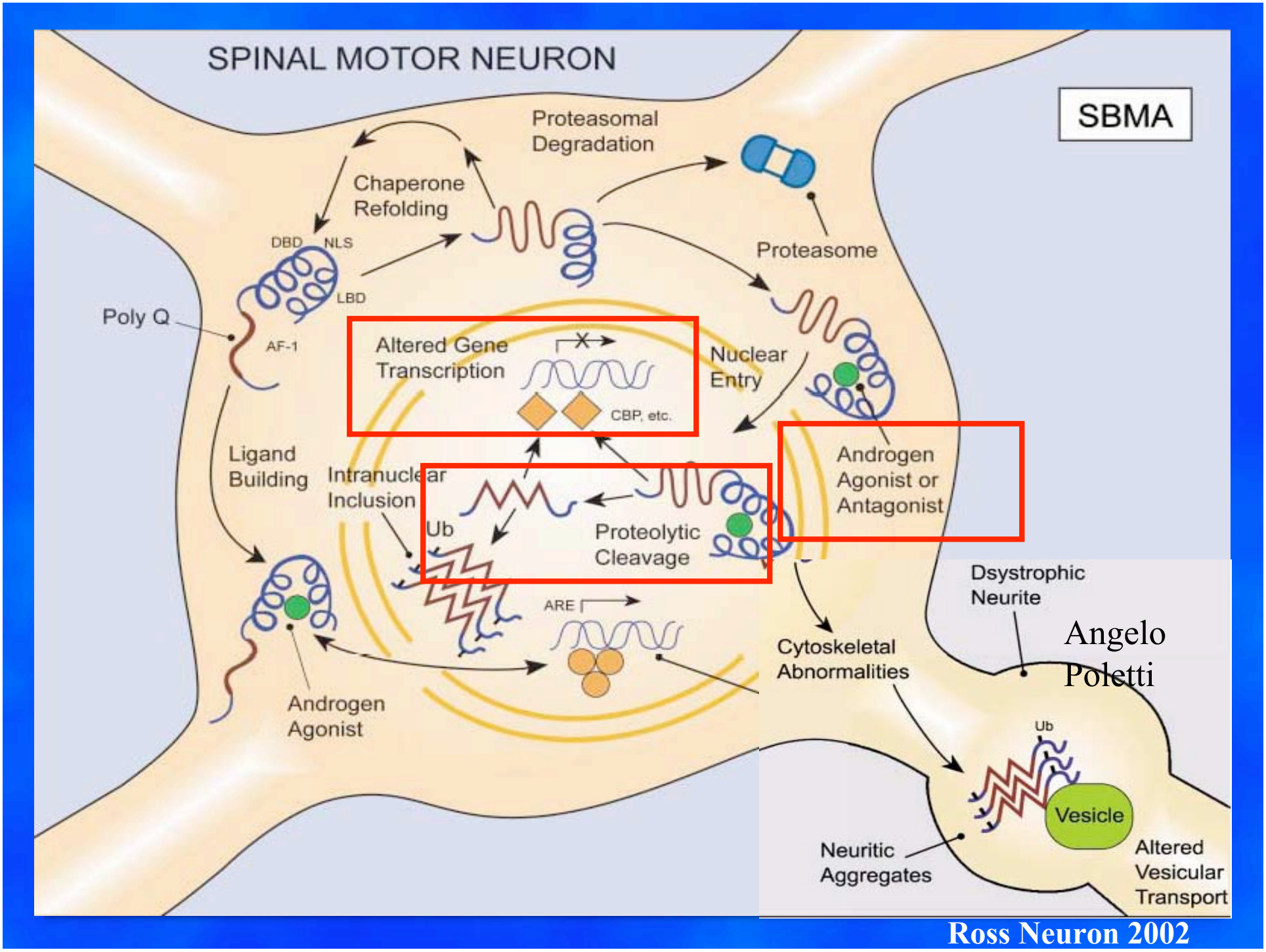
What is the mechanism of motor neuron dysfunction and loss?


SPINAL MOTOR NEURON
SBMA
Proteasomal Degradation
Chaperone Refolding
DBD
NLS
LBD
Poly Q
AF-1
Proteasome
Altered Gene Transcription
CBP, etc.
Nuclear Entry
Ligand Building
Intranuclear Inclusion
Ub
Proteolytic Cleavage
Androgen Agonist or Antagonist
ARE
Androgen Agonist
Dsystrophic Neurite
Angelo Poletti
Cytoskeletal Abnormalities
Ub
Vesicle
Neuritic Aggregates
Altered Vesicular Transport
Ross Neuron 2002

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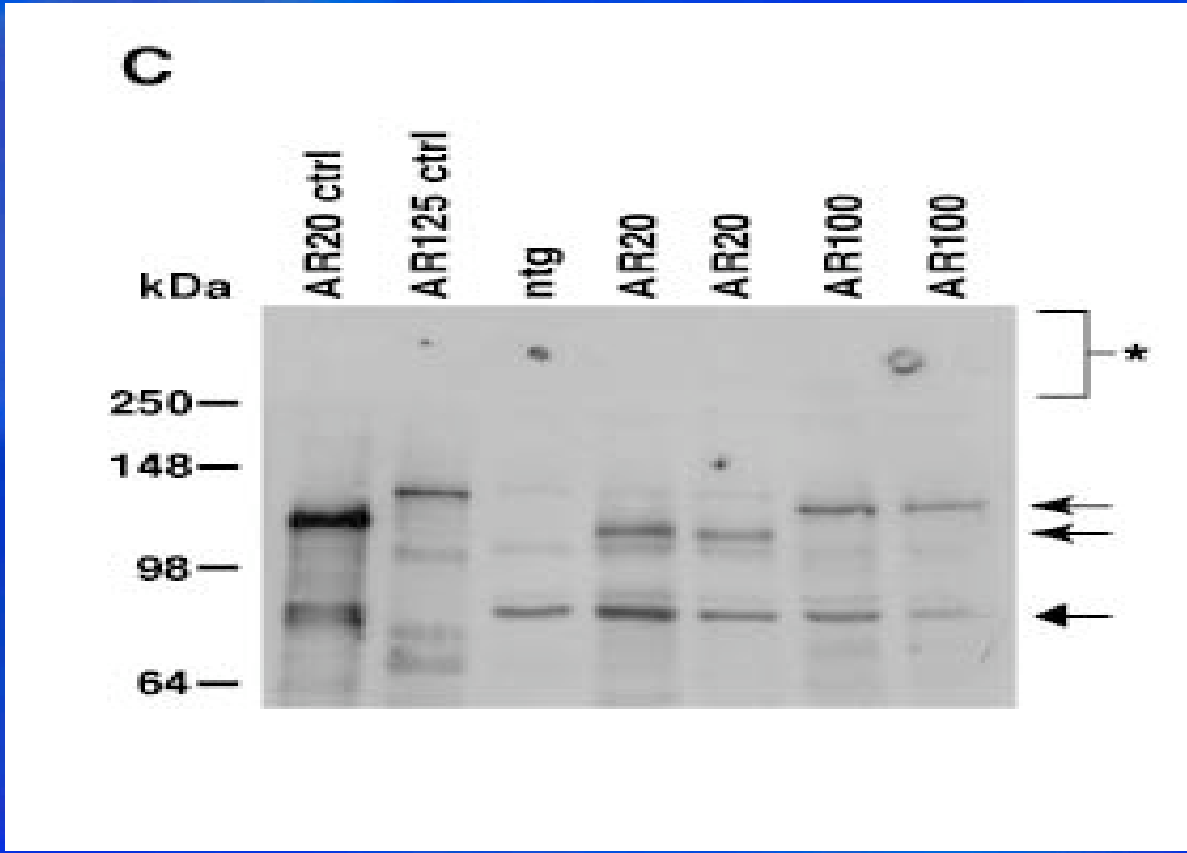
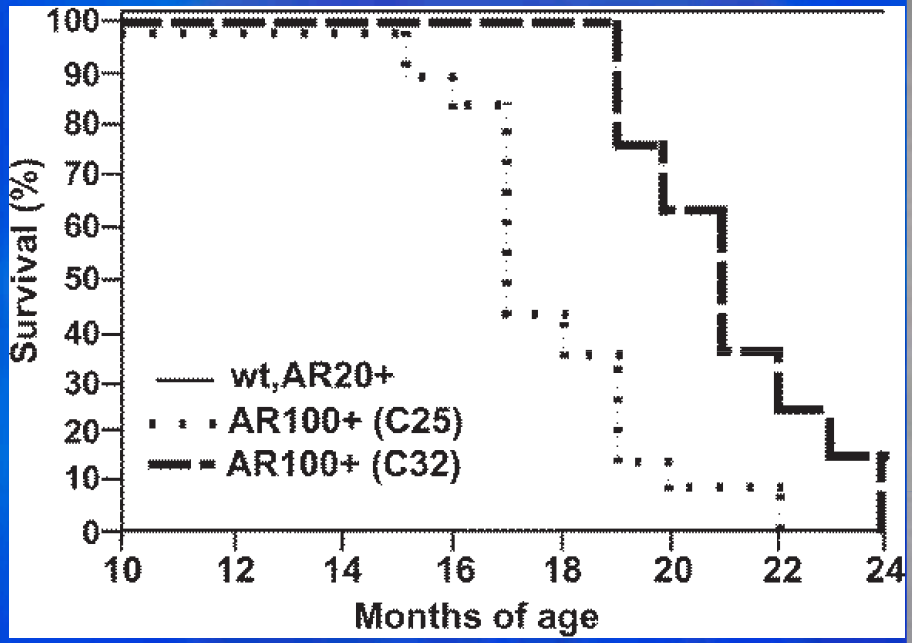
Generation of SBMA mouse model that recapitulates disease phenotype

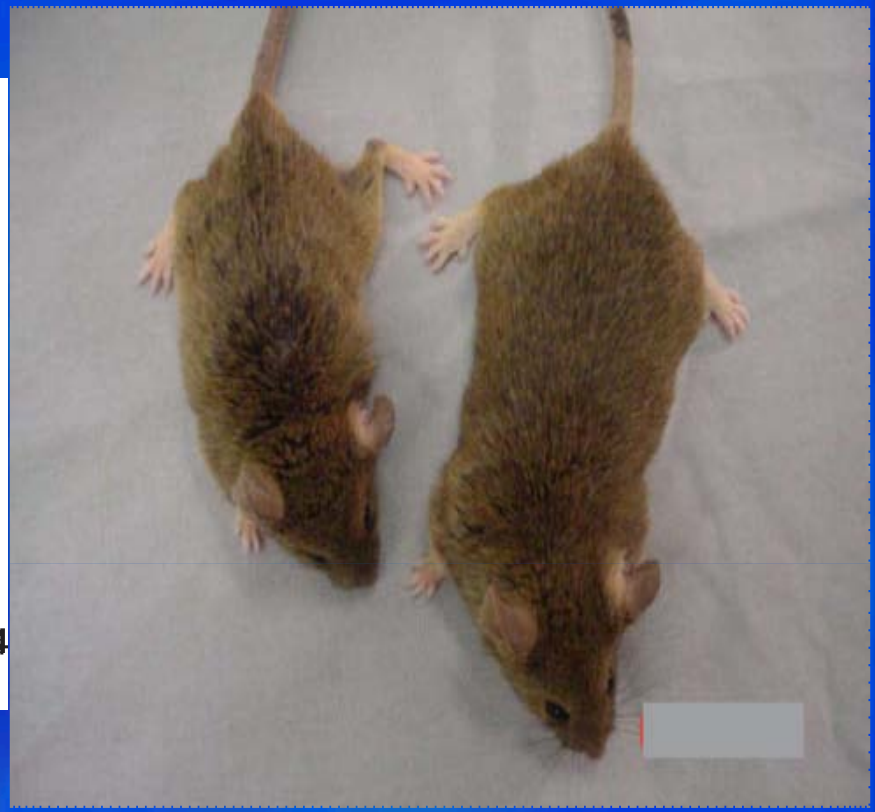
SBMA-Mouse Model that Recapitulates Disease

Human AR expressed in Spinal Cord of YACs



SBMA-Mouse Model that Recapitulates Disease

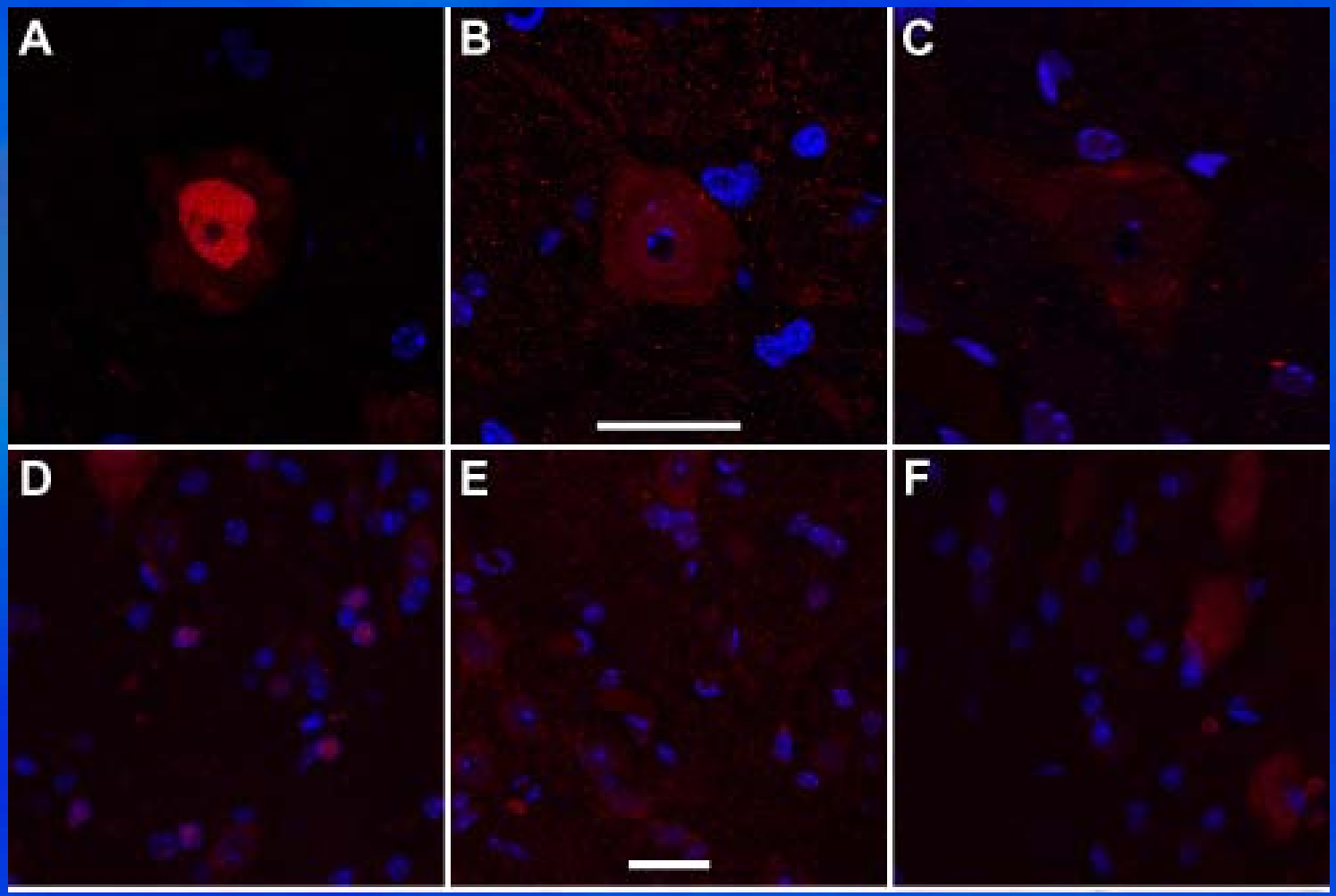




10 month age

SBMA-Mouse Model AR100 is in the Nucleus

AR100 Control



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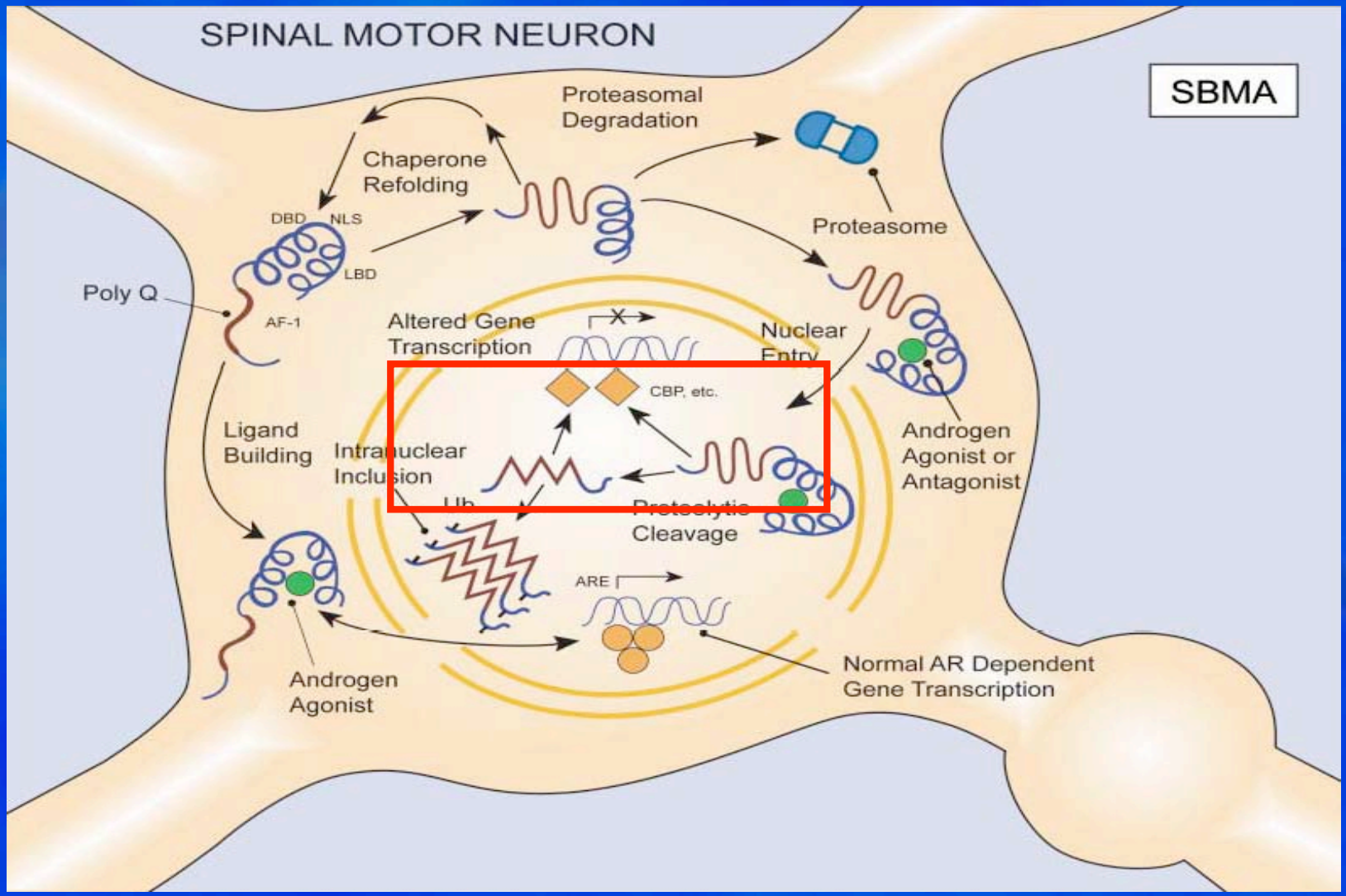
Transcriptional Dysregulation-SBMA Neurotoxicity



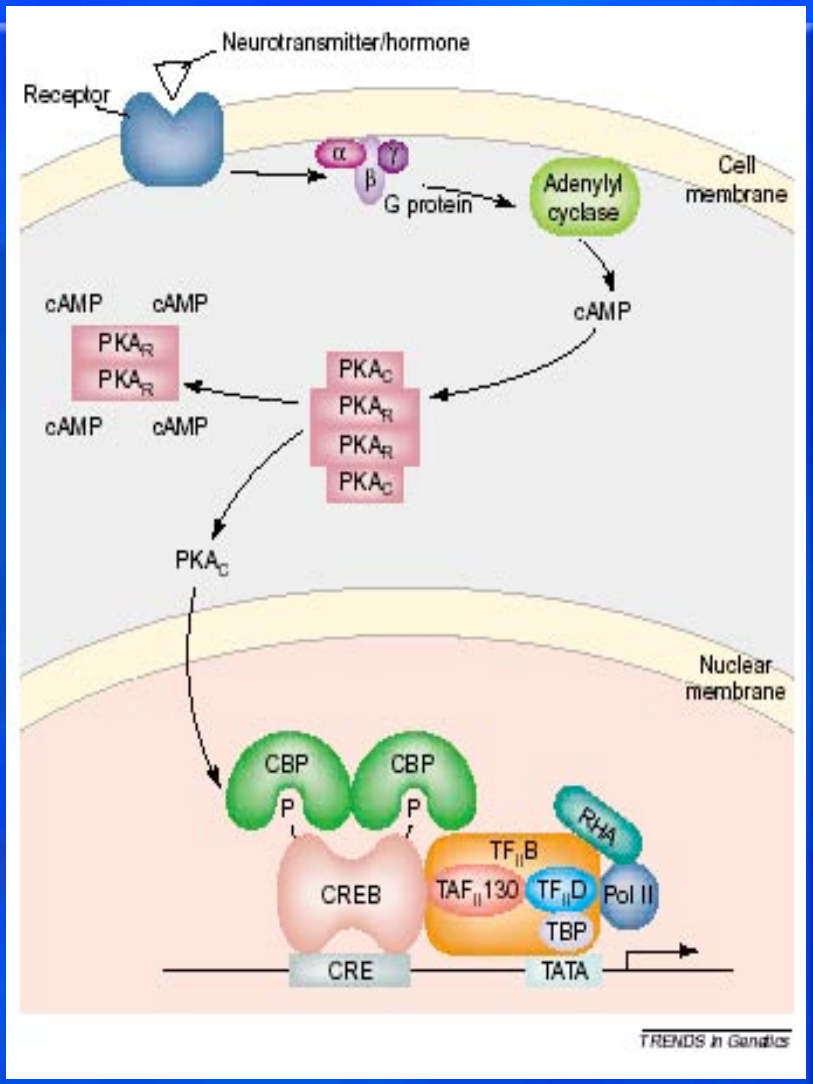
Ross Neuron 2002

Studies of Transcription in PolyQ Disease Models

Steffan…Thompson: CBP and p53-fly models
Paulson: CBP
McCambell…Fischbeck: CBP
Wyttenbach…Rubinsztein: CBP
Nucifora…Ross:CBP/CREB-HD and DRPLA Tg
Shimohata…Tsuji: TAF 130
Li: Sp1
Dunah…Krainc: TAF 130 and Sp1--via soluble interactions?

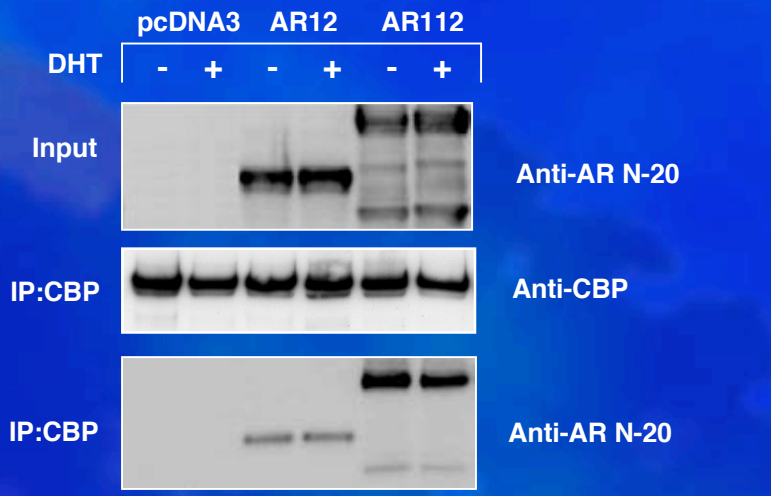
CBP and Neuronal Signaling

- CBP is activated by cell surface cAMP dependent signaling
- Other signaling pathways also converge on CBP
- CBP activates gene transcription, in part via Histone Acetyl Transferase (HAT) activity
- CBP-mediated transcription is important neuronal survival
- CBP is involved in activating a transcription factor known as hypoxia induced transcription factor (HIF1-α)- motor neurons

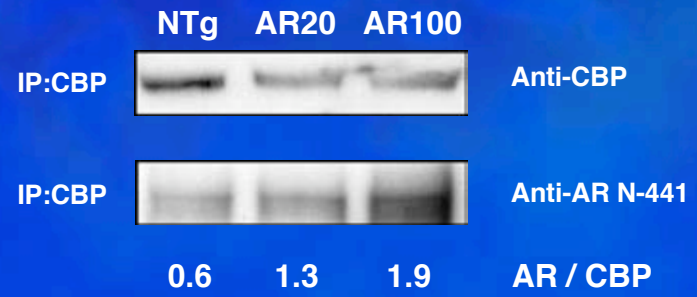


AR-PolyQ dependent binding of CBP

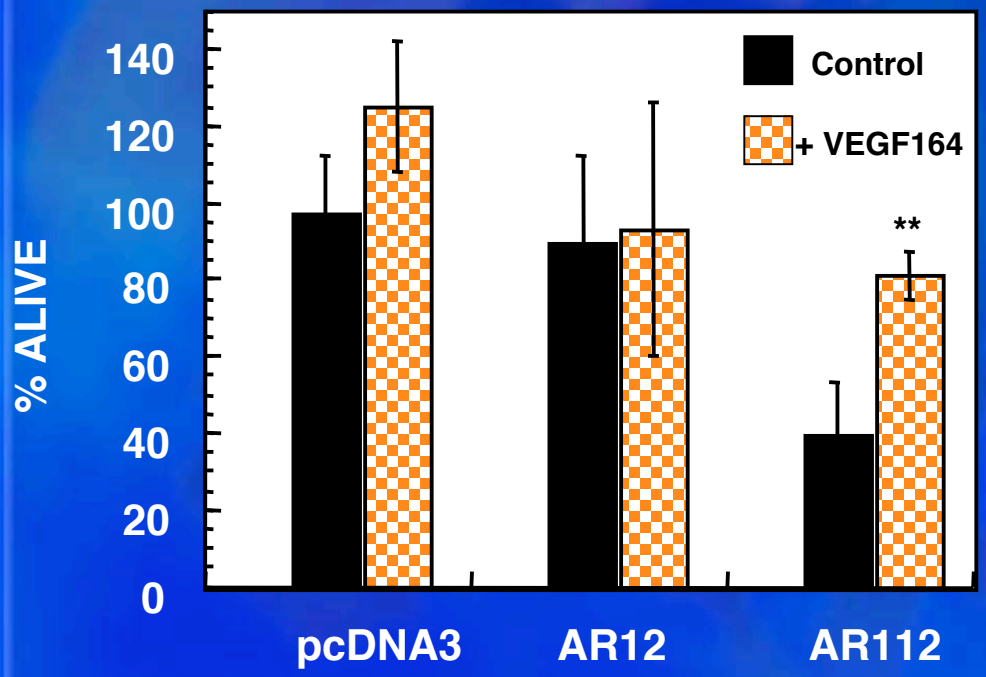
Motor neuron cell culture model

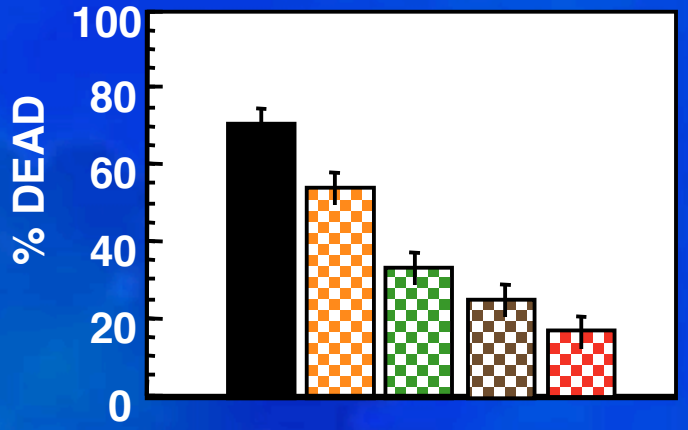


YAC SMBA model



VEGF Rescues Mutant Androgen Receptor Neuronal Death





AR112-untreated

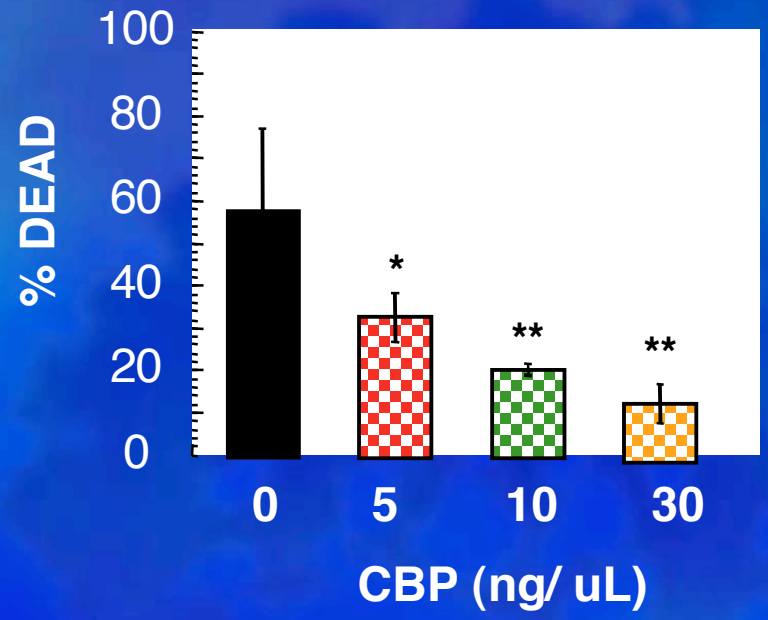
2.5 ng/ uL VEGF164

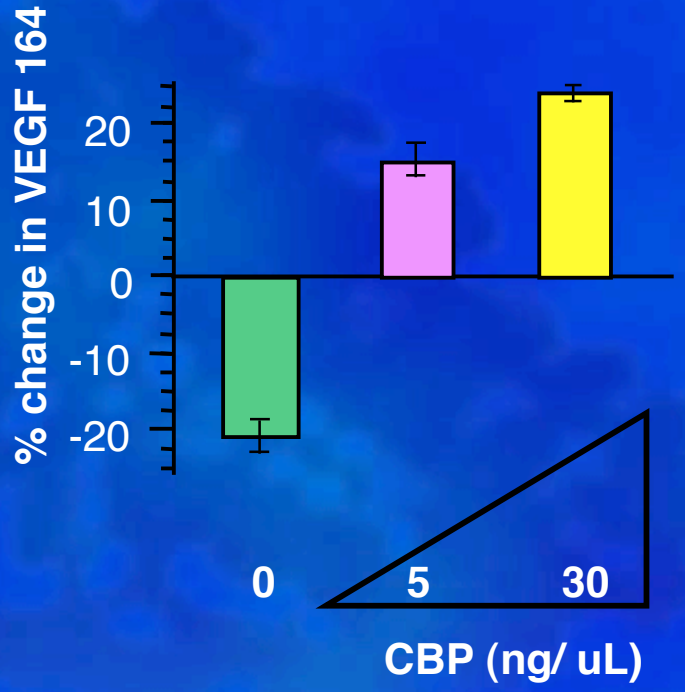
5.0 ng/ uL VEGF164

10 ng/ uL VEGF164

20 ng/ uL VEGF164

CBP restores VEGF 164 Levels Suggesting a Critical Role





CBP Rescues Mutant Androgen Receptor Neuronal Death

VEGF and Motor Neuron Survival

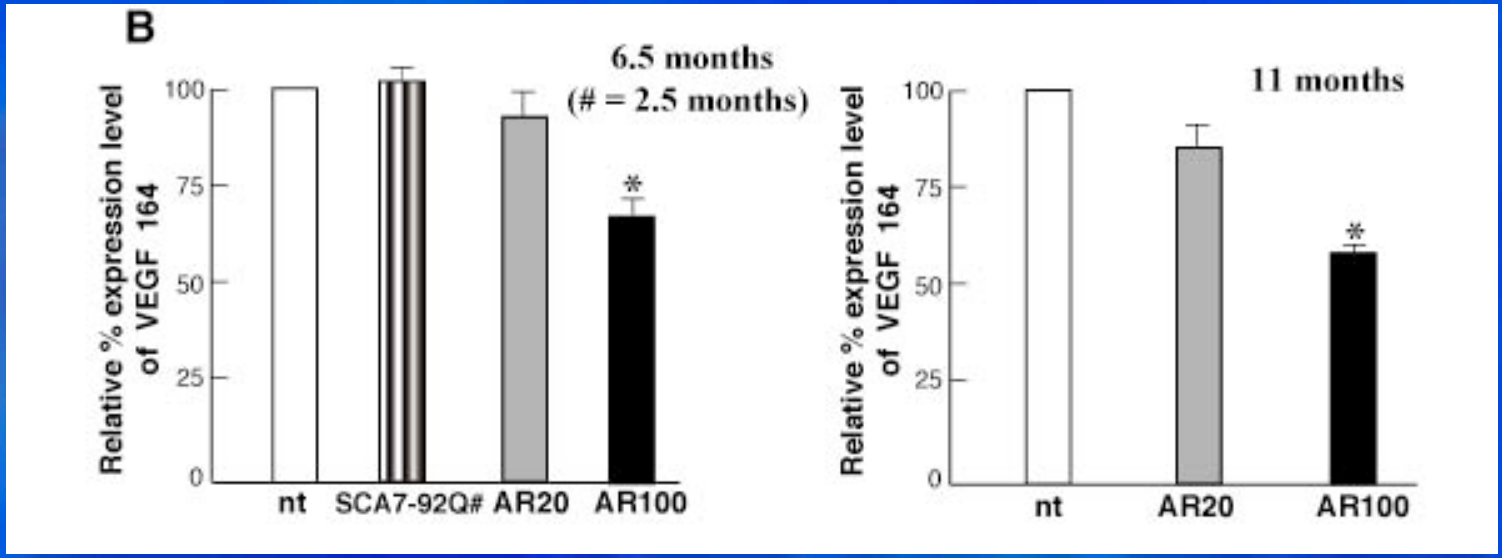
- VEGF receptor knockout embryonic lethal…

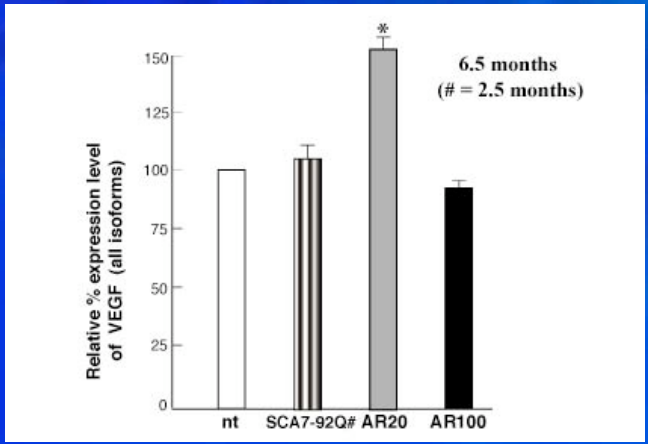
- VEGF required for motor neuron survival- Ooshuyse et al 2001 Nature Genet. 28, 131-138 Deleted hypoxia-response element from the Vegf promoter-ALS like phenotype with 30% reduction in VEGF levels in spinal cord

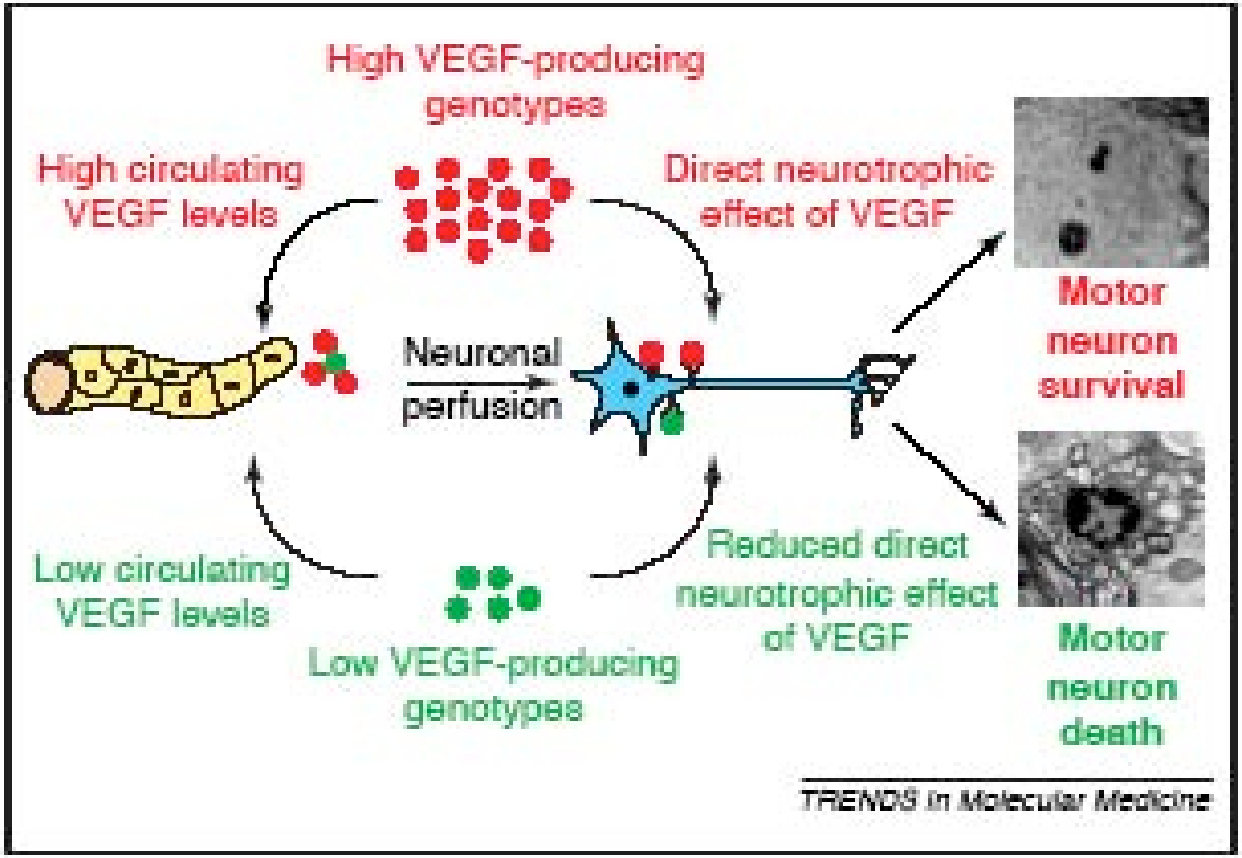
- CBP co-activator of hypoxia induced transcription factor (HIF-1) which regulates growth factor

- Test the hypothesis polyQ AR interferes with CBP action on HIF-1 and VEGF levels in the spinal cord

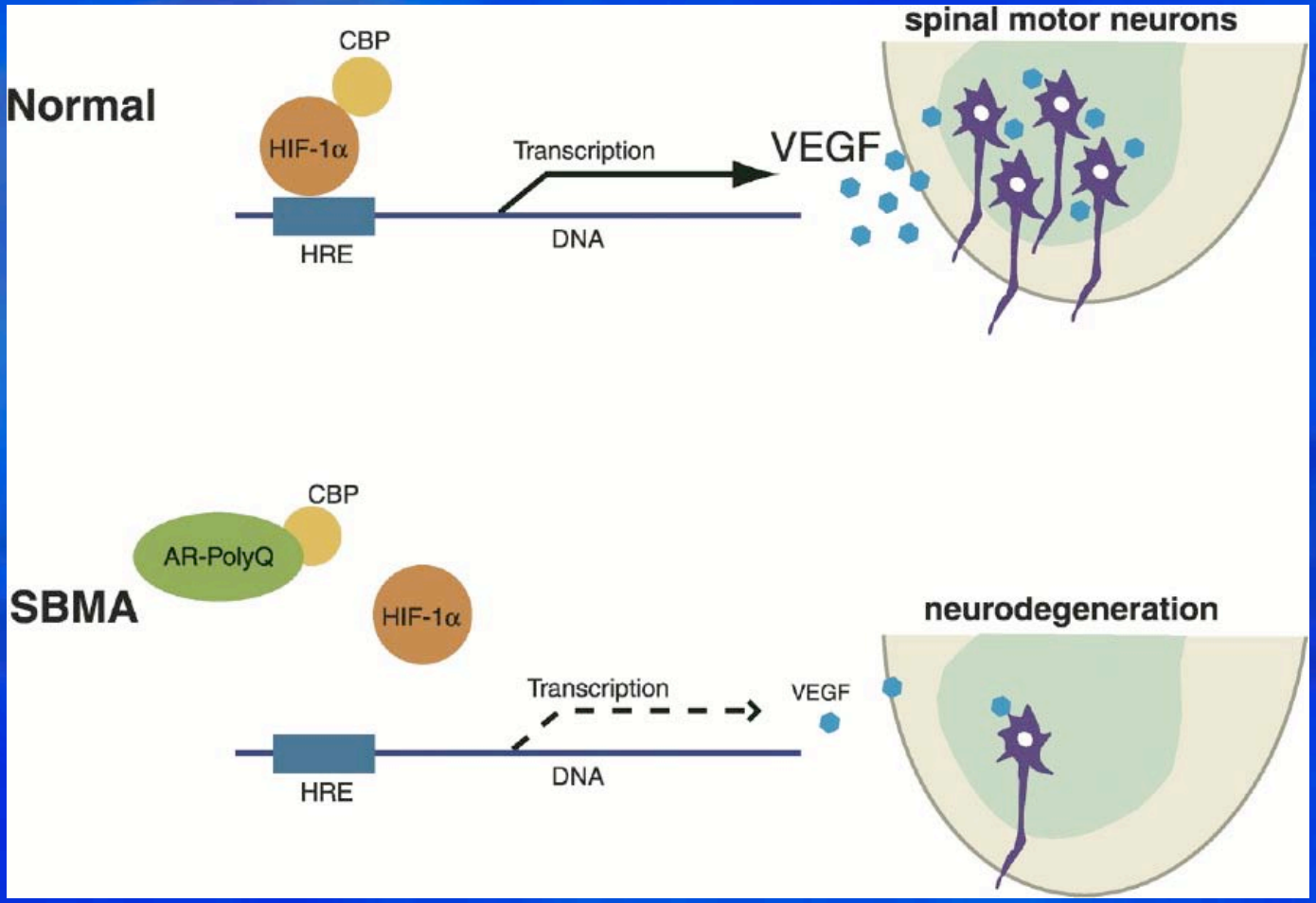
VEGF 164 LEVELS ARE ALTERED IN YAC SBMA TG MICE





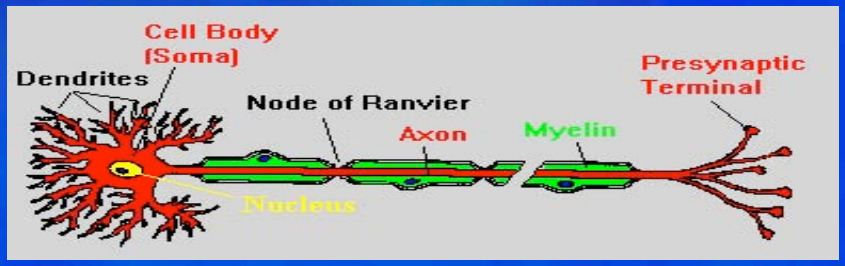

High VEGF-producing genotypes
High circulating VEGF levels
Direct neurotrophic effect of VEGF
Motor neuron survival
Neuronal perfusion
Reduced direct neurotrophic effect of VEGF
Low circulating VEGF levels
Low VEGF-producing genotypes
Motor neuron death
TRENDS in Molecular Medicine

SBMA-Transcriptional Dysregulation



Role of VEGF in Neuroprotection-Motor Neurons

- Deletion of hypoxia-response element from Vegf promoter develop ALS like syndrome.
- Cross-breeding these mice with fALS mice $SOD1^{G93A}$ accelerate phenotype.
- Variations in the human VEGF promoter/leader sequence, which is associated with reduced levels of circulating VEGF also confer increased risk of ALS.
- VEGF reduces glutamate excitotoxicity and production of free radicals.
- VEGF link to motor neuron disease in general provides prospects of new mechanistic insights and treatment of motor neuron disease.
- Mechanism of action not known…
  trophic support of motor neurons?



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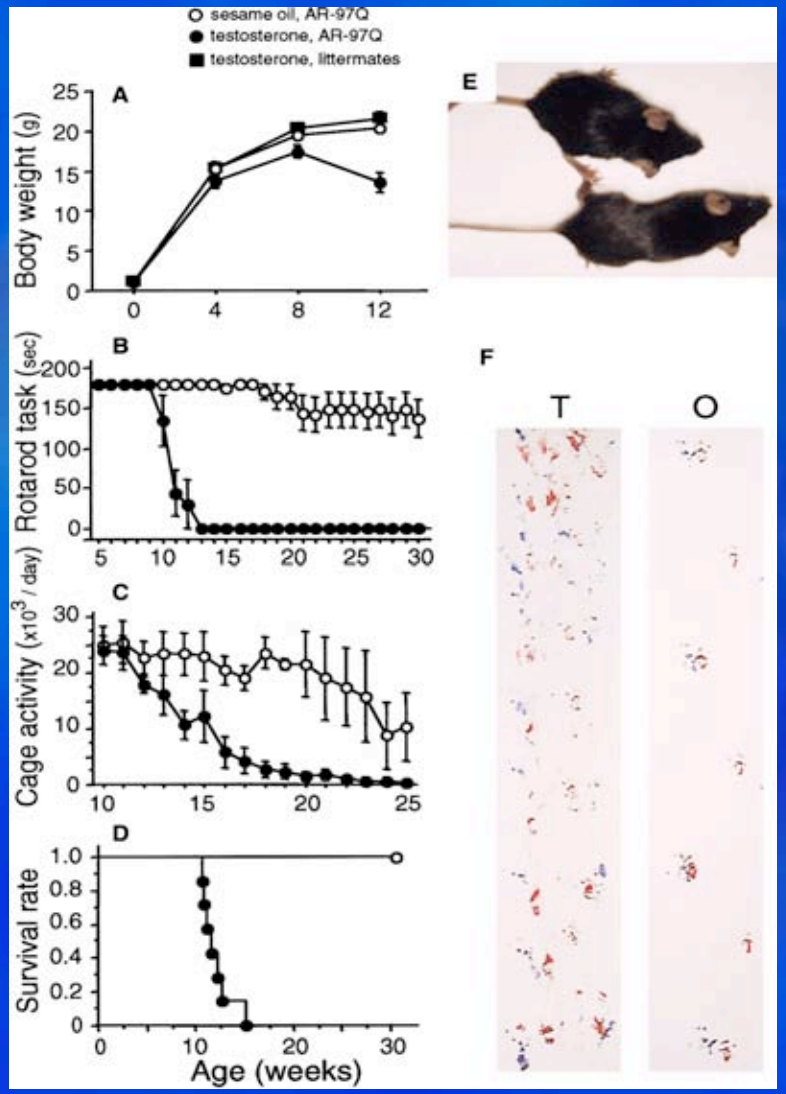
Castration Prevents the Kennedy's Disease



- Gen Sobue Neuron 35, 843-854, 2002.

- Leuprorelin-lutenizing hormone-releasing hormone angonist reduces androgen levels in the testis-medical castration-prevents nuclear translocation of AR

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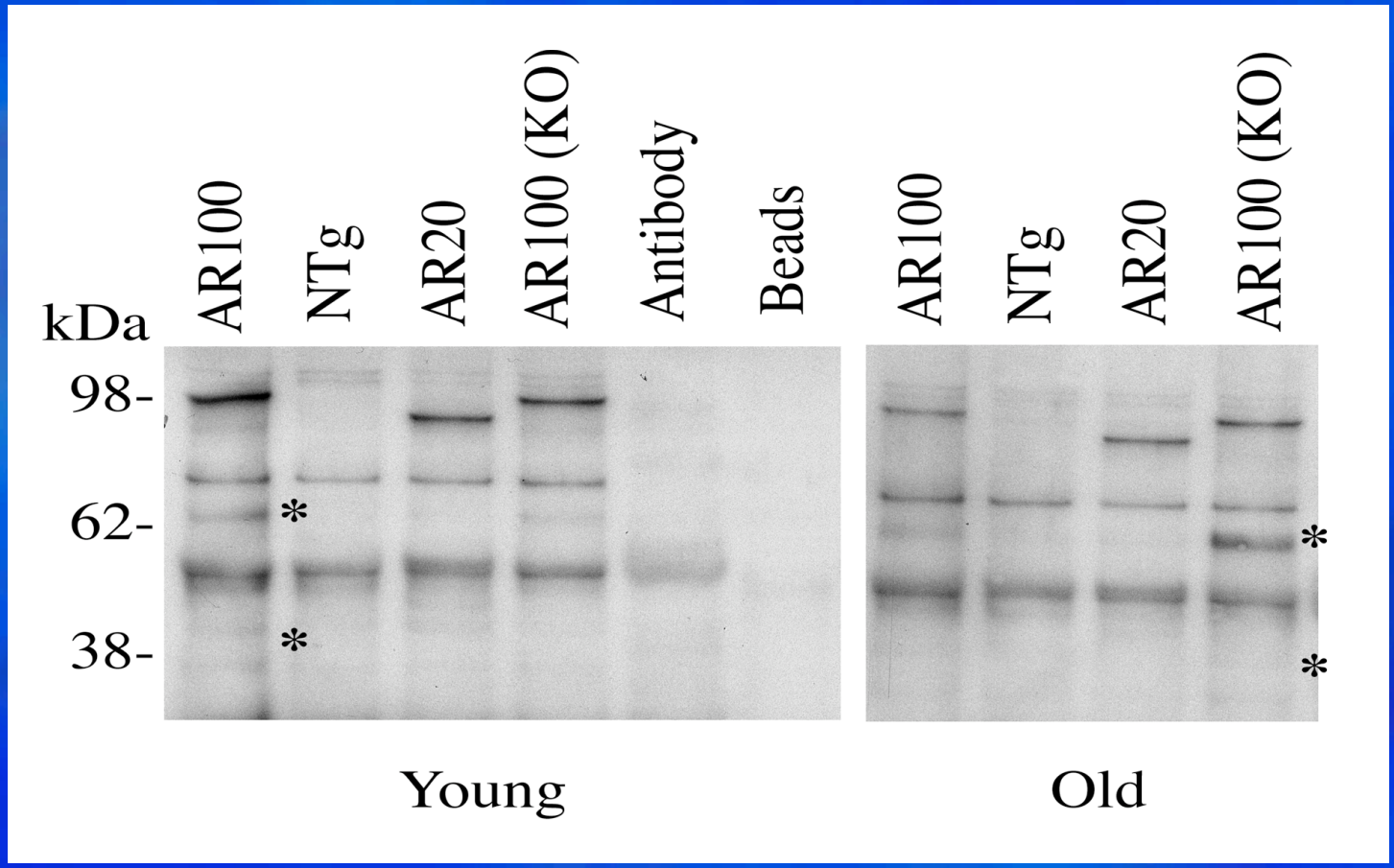
What is the role of testosterone in SBMA? Hypothesis: Androgen receptor fragments required for transcriptional dysregulation

Evidence for the Role of Proteolysis in SMBA

- Truncated fragments found in SMBA post-mortem tissue.
- Generation of transgenic model using endogenous human AR promoter and first exon of AR- rapid disease progression. Full-length AR models slow disease progression.
- Caspases cleave androgen receptor.
- Common properties to all polyQ diseases.
- Testosterone results in a polyQ dependent increase in proteolysis.

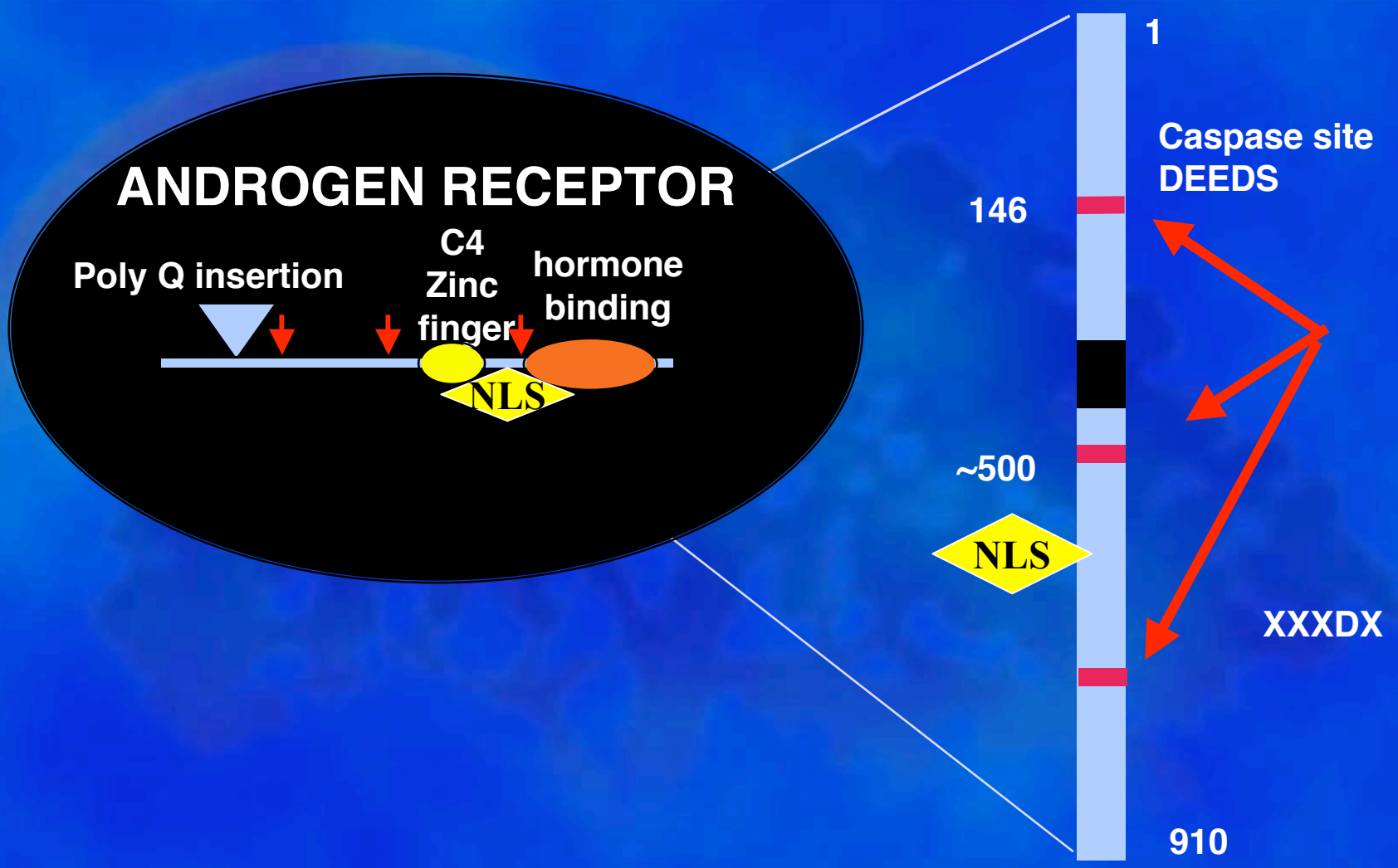
Cleavage

Evidence for the Role of Proteolysis in YAC100

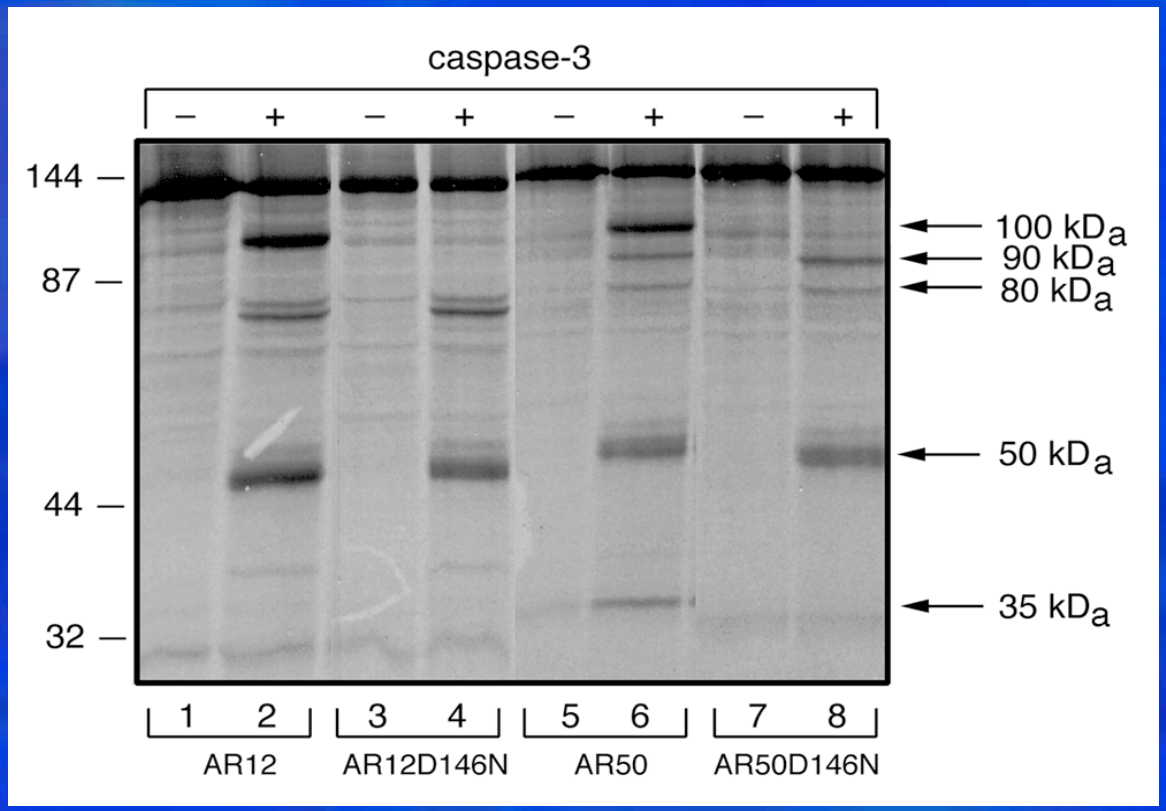


*polyglutamine dependent N-terminal fragment

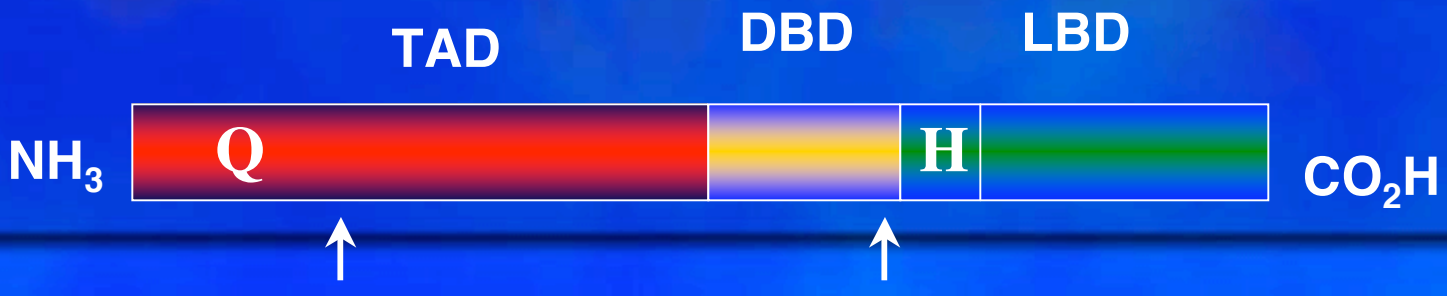
Proteolysis of Androgen Receptor



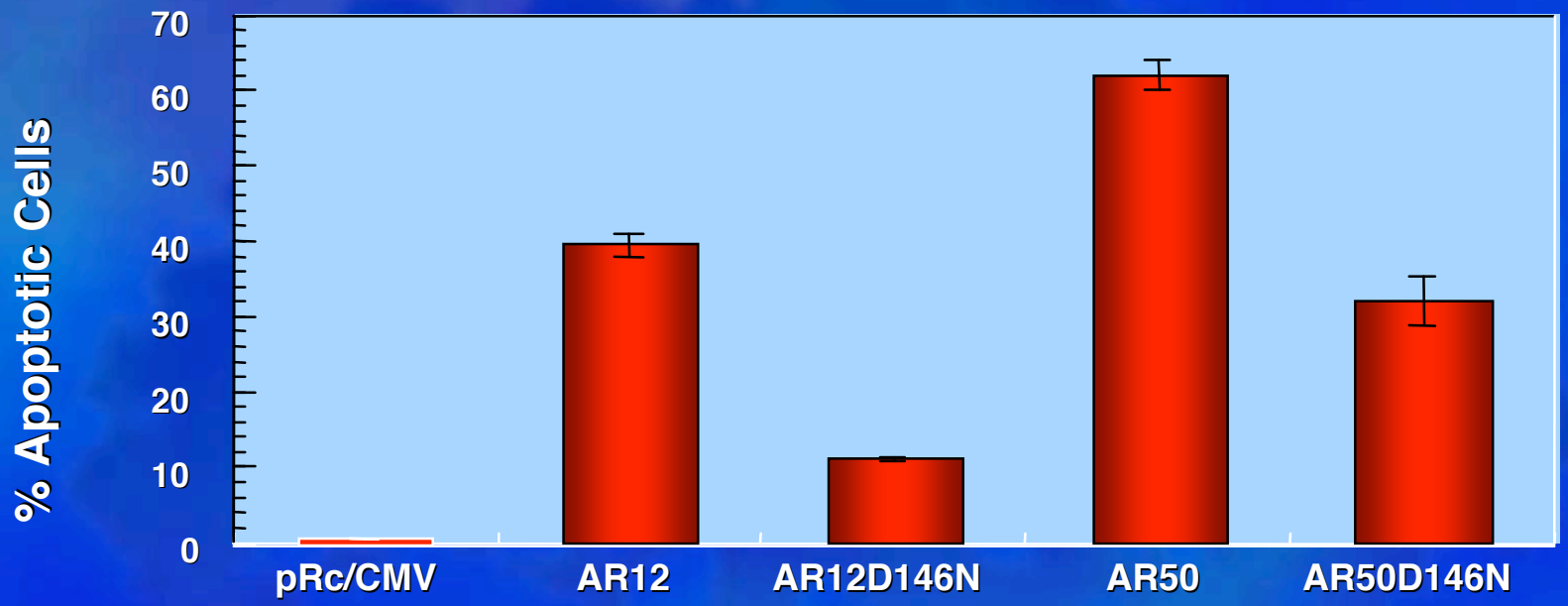
Androgen Receptor is Cleaved by Caspases



polyQ fragments



Caspase Resistant AR Reduces Cellular Toxicity

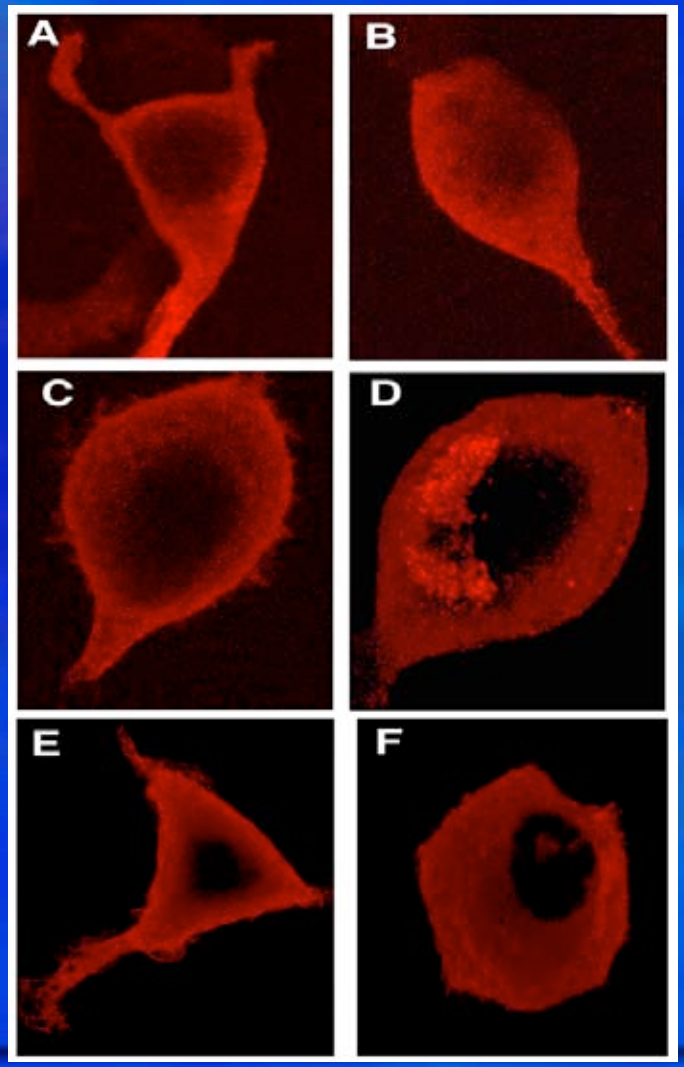


Aggregate Formation in SBMA AR

AR12

AR5O

AR12
D146N
+TAM

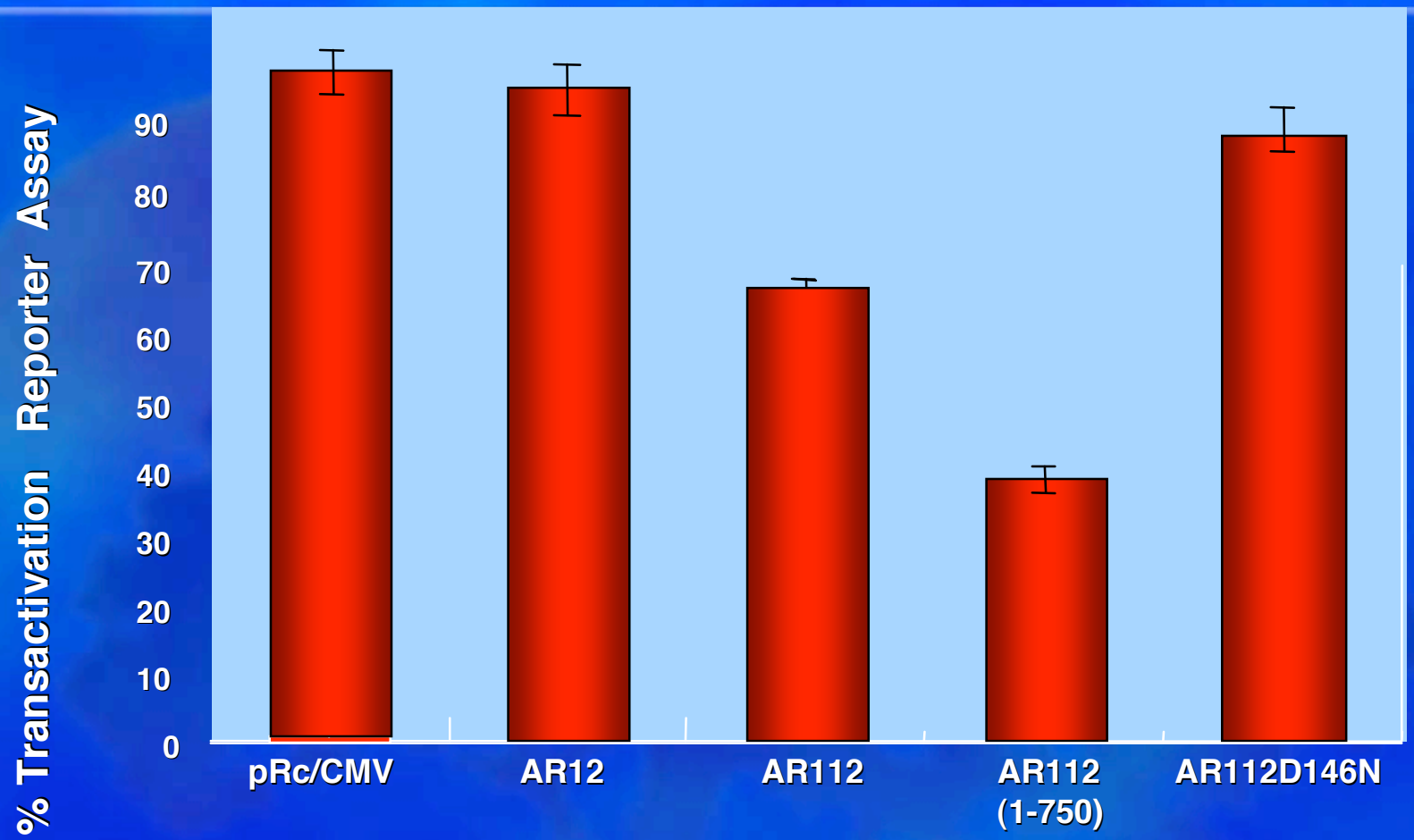


AR12
+TAM

AR5O
+TAM

AR50
D146N
+TAM

Caspase Resistant AR-Transcriptional Dysregulation



Generation of SBMA Transgenic Mouse Models

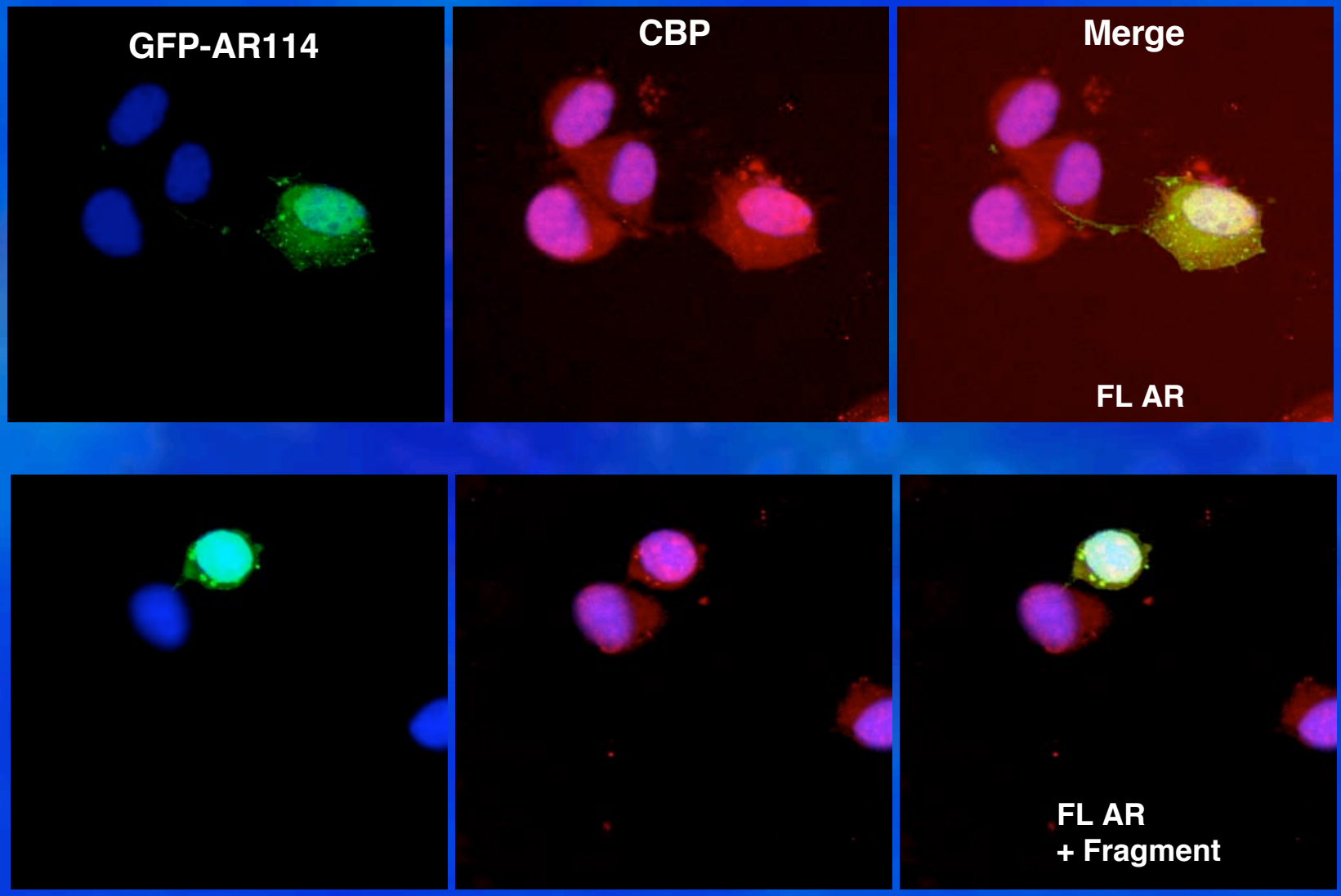
AR12

AR112

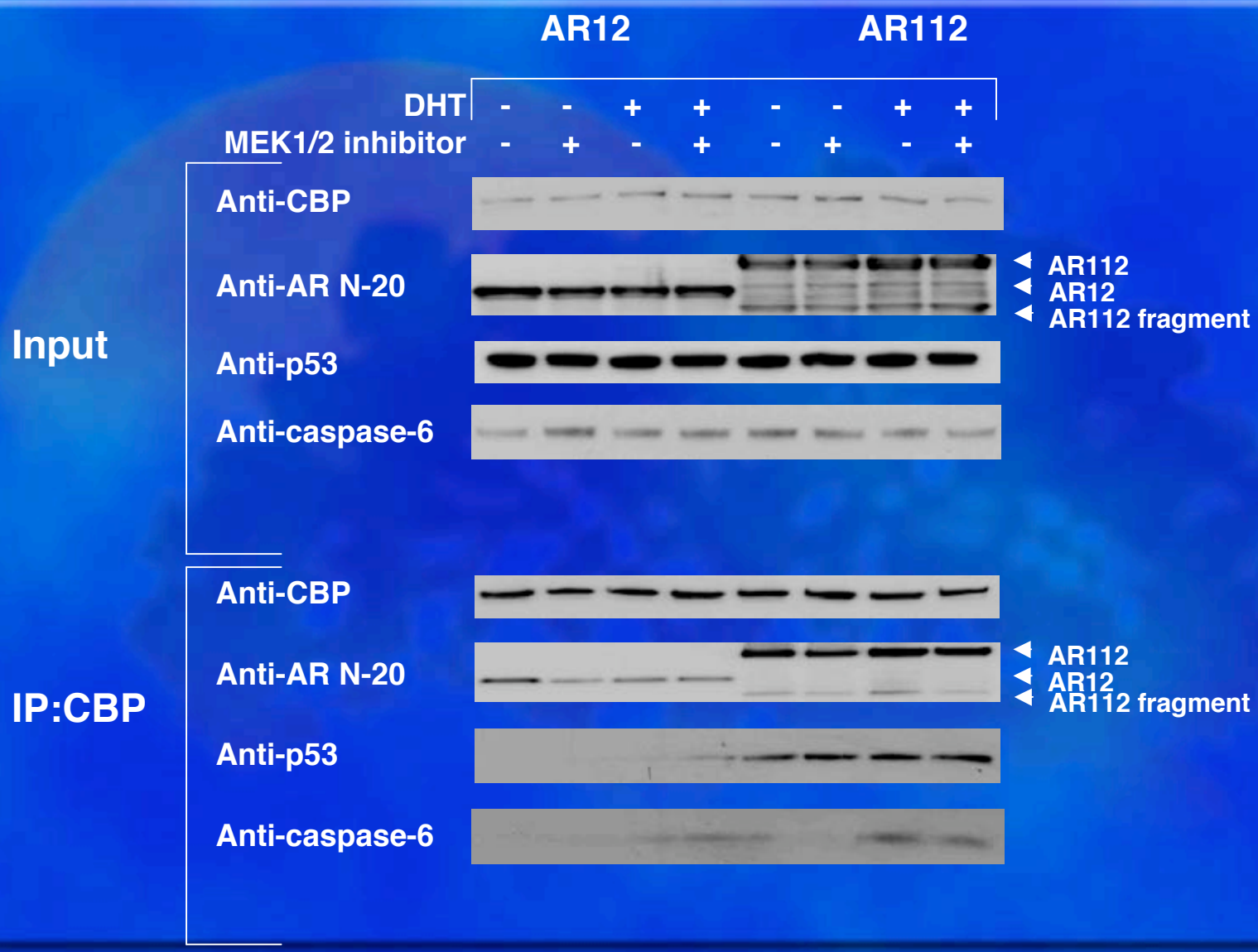
AR112 D146N

CBP, p53, Caspase form Complexes-Dysregulation

PolyQ EXPANSION IN AR ALTER LOCALIZATION OF CBP



FL AR COMPLEX WITH CBP, p53 AND CASPASE-6



Conclusions:

1) SBMA is a motor neuron disease-testosterone plays a critical role in disease pathology and progression

2) SBMA treatment
   - androgen-blockage drugs used to treat prostate cancer
   -growth factors that are required for motor neuron survival-VEGF
   -protease inhibitors block production of toxic fragments

3) VEGF levels play an important role in motor neuron diseases such as fALS and SBMA

The Ellerby Lab

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