

Mary E. Brunkow, PhD



Nobel Lecture

7 December 2025

FOXP3/Scurfin:

A Key Driver of Peripheral
Immune Tolerance





Larry Sandler
University of Washington
Dept. of Genetics

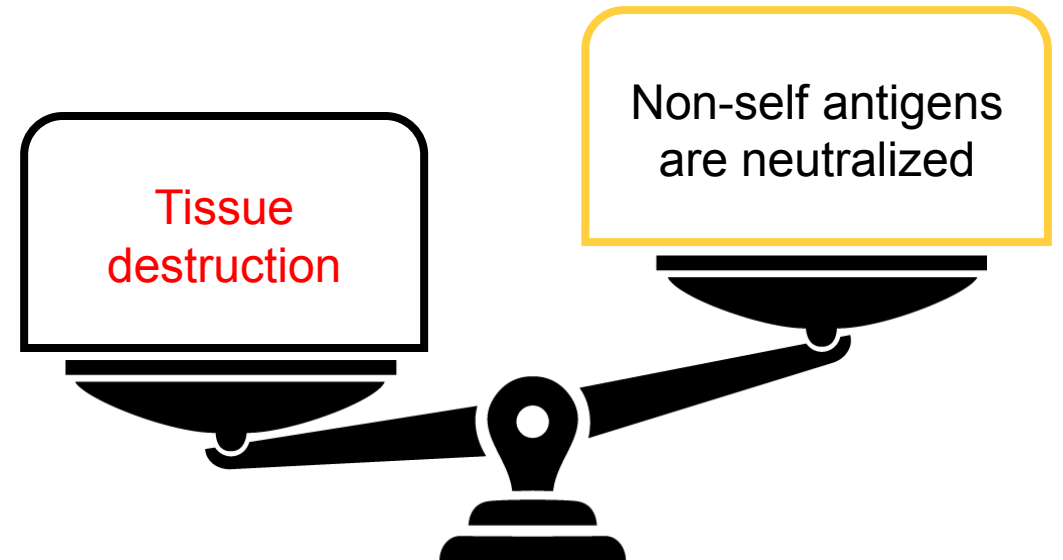


Shirley Tilghman
Princeton University
Dept. of Molecular Biology

Immune Tolerance

Healthy

Autoimmune disease,
transplant rejection, etc.



IMMUNE
HOMEOSTASIS

IMMUNE
IMBALANCE

Origins of the scurfy mouse



William and Liane Russell



Located in eastern Tennessee, USA
est. 1943 as part of the Manhattan Project

Mammalian Genetics Section was established to study:

- Hereditary effects of radiation (fallout from nuclear tests)
- Radiation-induced mutation rates



Genetics and Pathology of the scurfy Mouse



scurfy:

- **Spontaneous mutation**
(i.e., not part of the radiation exposure cohort)
- **Observed in 1949**
Maintained for ~40 years before pathology was described!
- **First X-linked mutation in mouse**
(no obvious phenotype in female carriers)

Visible Phenotypes:

Hunched posture
Runting
Squinted eyes
Scaly skin
Cachexia

Internal Phenotypes

Splenomegaly
Hepatomegaly
Enlarged lymph nodes
Small thin thymus

Genetics and Pathology of the scurfy Mouse



Male mice only:

Severe lymphoproliferative disorder is lethal by ~3 weeks

- Mediated by CD4+ T cells
- Cytokine elevation
- Down regulation of T cell activation is defective >> autoimmunity



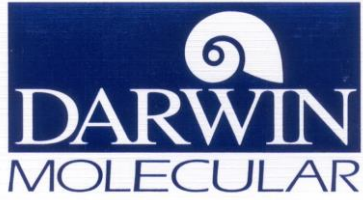
**Virginia
Godfrey**
DVM, PhD



**John Erby
Wilkinson**
MS, DVM, PhD

Gene-Driven Drug Target Discovery

“DNA Sequences to Drugs through Molecular Evolution”



- Launched in 1993 – Bothell, Washington, USA
- Phenotype-driven target discovery
- Target areas: Autoimmune disease, Cancer, AIDS
- Interactions with Oak Ridge National Lab >> interest in scurfy mouse
- Acquired by Chiroscience (1996), then Celltech (1999), both in the UK
- Closed 2004

Founders:

David Galas, PhD

Leroy Hood, MD, PhD

Ronald Cape, MBA, PhD

Lawrence Loeb, MD, PhD

George Rathmann, PhD

Julius Rebek, Jr., PhD

Mark Pearson, PhD

Gerald Joyce, MD, PhD

Lloyd Cotsen, MBA

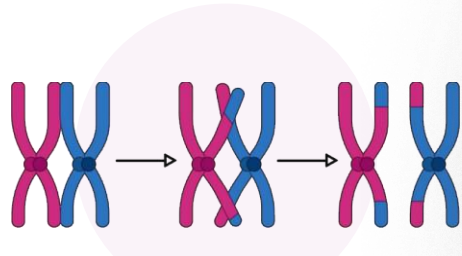


Two-Pronged Approach to Cloning scurfy

1 Genetic mapping to refine scurfy interval

Let the mice do the work for you!

- Brought scurfy mice from ORNL to Darwin
- Set up large backcross experiment to capture new recombination events



Sheet1

	<u>DXWas70</u>	<u>123</u>	<u>101</u>	<u>26</u>	<u>55</u>	<u>Gata1</u>	<u>124</u>	<u>161</u>	<u>136</u>	<u>Otc</u>	<u>27</u>	<u>89</u>	<u>102</u>	<u>162</u>	<u>Timp</u>
46848	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
49353	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
N3	C	C	C	C	C	C	C	C	<u>129</u>	C	C	C	C	C	S
49719	C	S	S	S	S	S	S	S	S	S	S	S	S	S	S
51555	C	C	C	S	S	S	S	S	S	S	S	S	S	S	S
51746	S	C	C	C	C	C	C	C	<u>129</u>	C	C	C	C	C	C
52247	S	S	S	S	S	S	S	S	S	C	C	C	C	C	C
52607	S	S	S	S	S	S	S	S	S	C	C	C	C	C	C
51046	S	S	S	S	S	S	S	S	S	S	S	S	S	S	C
51616	S	S	S	S	S	S	S	S	S	S	S	S	S	C	C

"underlines" indicate identical segregation patterns
non-recomb.

ORNL + DARWIN

DXWas70 [DXMit101, 123] [GFI, Tfe3, Wasp, Syp, Cybb, DXMit26, 55, 136, 124, 161] [Otc, DXMit27, 89, 102] DXMit162 Timp

EUe1B + y. Boyd

DXWas70₁₀₁ Nph1 (e1e1.5) DXMit123 Syp₅₅ Mit26 Tfe3 Gata1 Mit136 Xk Cybb₁₂₄ Otc₈₉ Mit162 Mit54

Wasp

Wasp1

Gap Gap

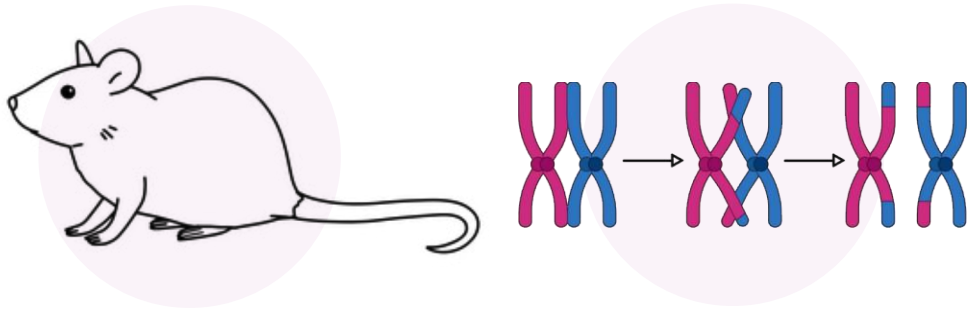
Page 1

Two-Pronged Approach to Cloning scurfy

1 Genetic mapping to refine scurfy interval

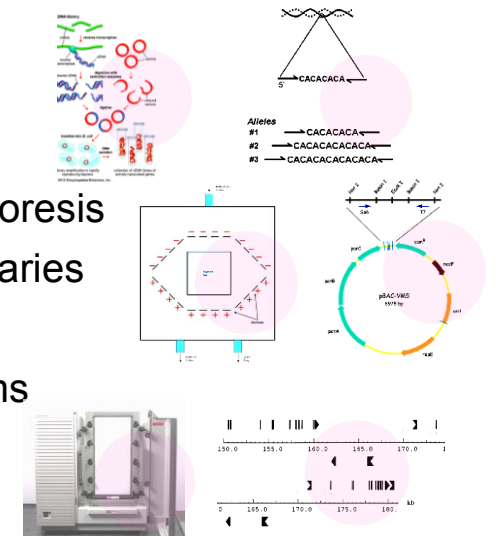
Let the mice do the work for you!

- Brought scurfy mice from ORNL to Darwin
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2 Physical mapping of scurfy interval

- Cutting-edge Genomics and Bioinformatics tools
- Highly accurate sequencing across >500kb interval
- cDNA libraries
- Polymorphic markers
- Pulsed field gel electrophoresis
- Artificial chromosome libraries
- Automated sequencing
- Gene prediction algorithms



| Finding the scurfy Mutation

My core team:



Eric Jeffery



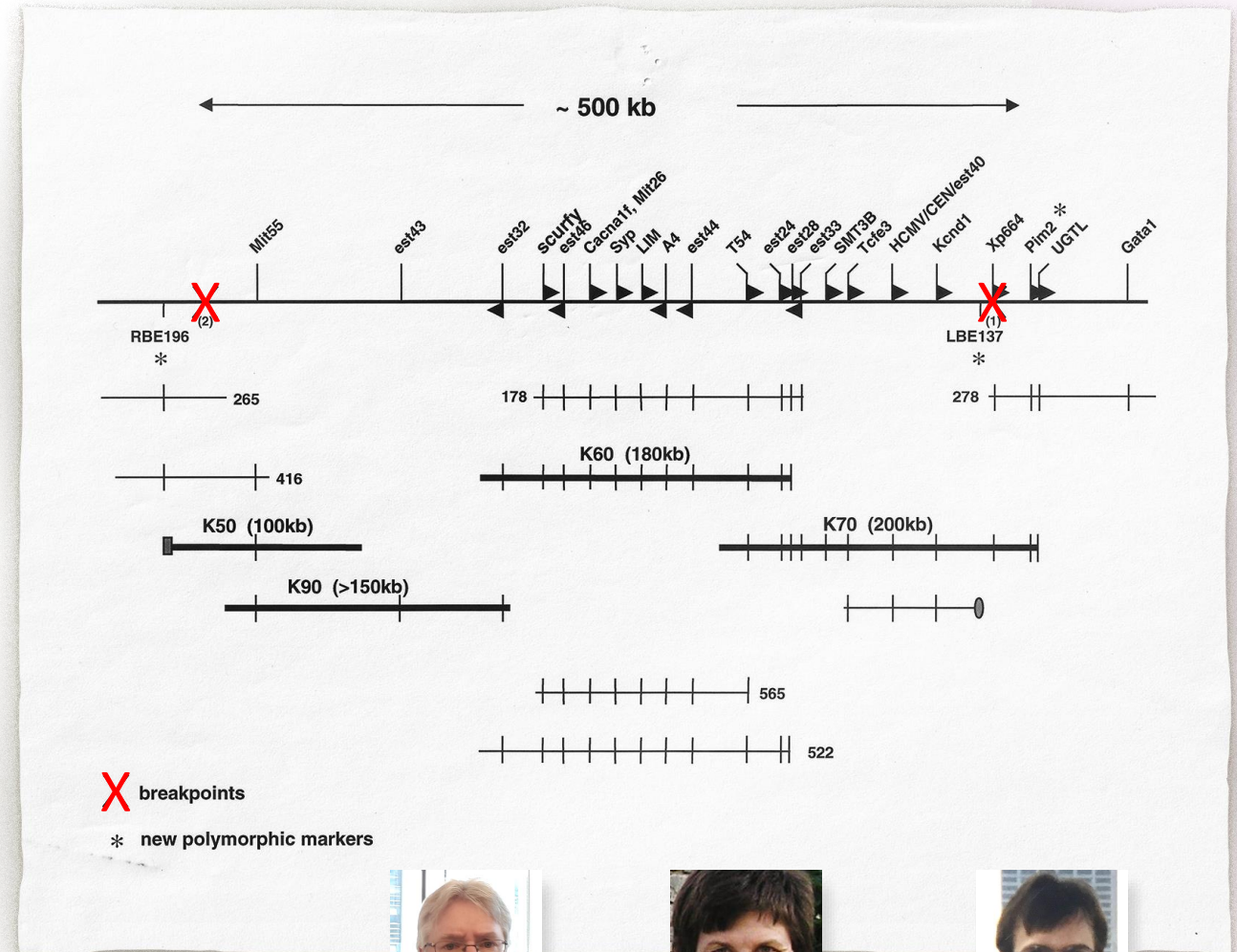
Kathryn Hjerrild



Bryan Paeper

Finding the scurfy Mutation

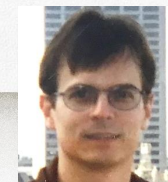
1. >500 kb BAC contig spanning critical recombination breakpoints (0.3-cM interval)
2. Highly accurate shotgun sequencing of tiling path BACs



Eric Jeffery



Kathryn Hjerrild

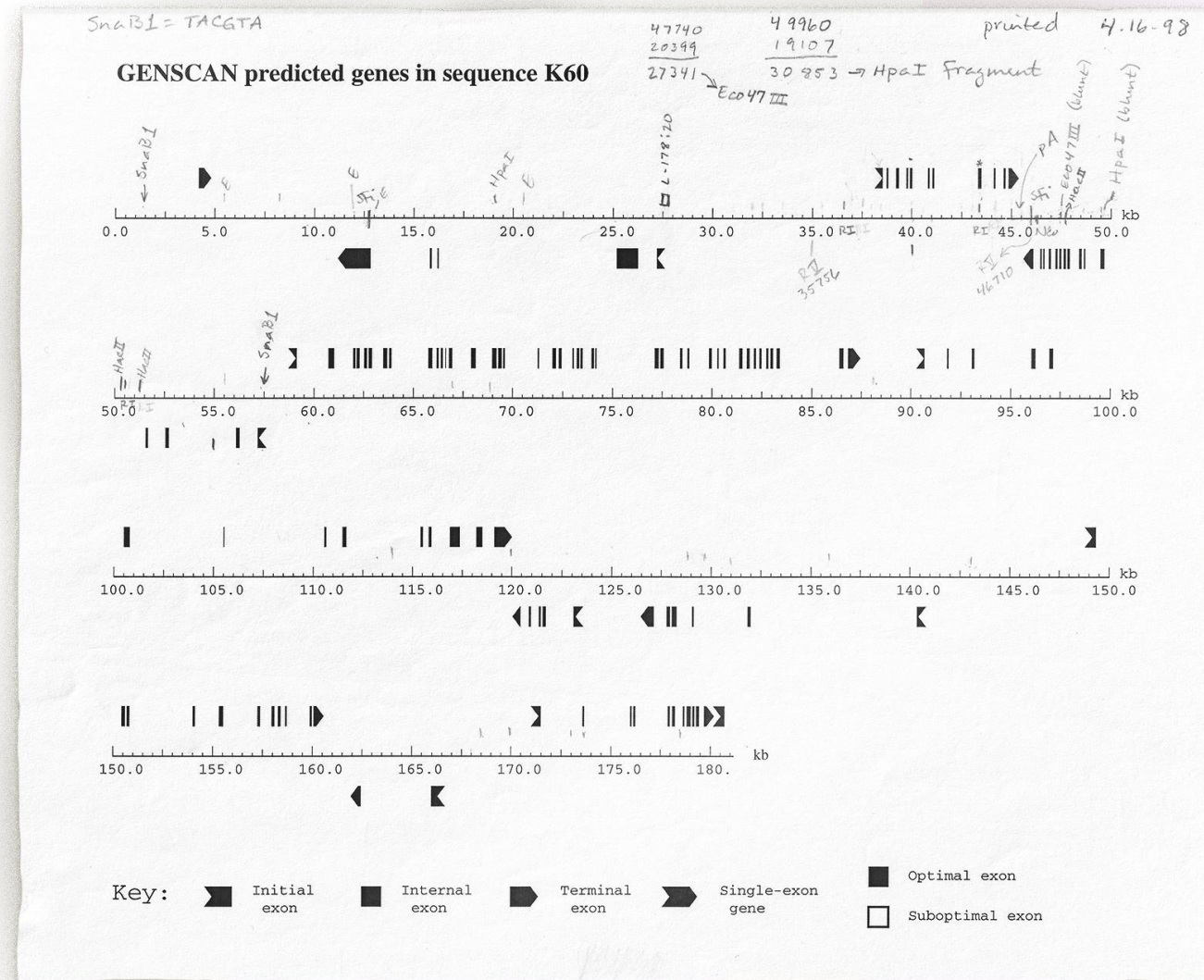


Bryan Paeper

Finding the scurfy Mutation

- >500 kb BAC contig spanning defining recombination breakpoints (0.3-cM interval)
- Highly accurate shotgun sequencing of tiling path BACs
- Gene identification: BLAST, GENSCAN, etc.
- 20 genes – known and novel

- | | | | |
|----------|-------------|--------------|--------------|
| 1. Syp | 6. Cacna1f | 11. AA273854 | 16. AF229635 |
| 2. Plm2 | 7. Plp1a | 12. AA030924 | 17. AW342118 |
| 3. Tcfe3 | 8. AF229644 | 13. Smt3h2 | 18. AF229642 |
| 4. mT54 | 9. AA124124 | 14. Lmo6 | 19. AF229645 |
| 5. Plp2 | 10. Kcnd1 | 15. AF229636 | 20. FKH |



Finding the scurfy Mutation

- >500 kb BAC contig spanning defining recombination breakpoints (0.3-cM interval)
- Highly accurate shotgun sequencing of tiling path BACs
- Gene identification: BLAST, GENSCAN, etc.
- 20 genes, known and novel
- scurfy vs wild-type sequence comparisons

1. Syp	6. Cacna1f	11. AA273854	16. AF229635
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TITLE *Results of sequencing scf. ORF 1/2, 2/4 fragments* PROJECT NO. 141 BOOK NO. 343

Work continued from Page 135

only obtained sequence of ORF from Jones seq 122 and seq 244. Seq 128 was just vector. A band seen at P. 135 was a PCR artifact. Seq 243 had both ORF 2 and 4 primer sequences present, but could not OR. Don't know how to explain that! Seq 244 sequence is shown below. This clone has a two "A" insertion as shown below which causes the protein product to terminate prematurely.

Seq. same as +/- insertion.

scf ORF 1/2, 2/4 Sequencher™ "ORF applied fr Genscan" ORF-4 probably not present, seq not good here

18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35

CTANCCACTG CTGCAAAAT GAGTCTGCA AGNCGCTGG TTGTGAGAAG
? H C W A N G V C K ? P P G C E K

GTCTTCGAGG AGCCAGAAGA GHTTCTCAAG CACTGCCAAG CAGATCATCT
V F E E P E E ? L K H C Q A D E L

CCTGGATGAG AAGGCAAGG CCCAGTGCCT CCTCCGAGA GAAGTGTGC
L D E K G K A Q C L L O R E V V

AOTCTCTGGA GCAGCAGCTG GAGCTGGAAA AGAGAAGCT GGGAGCTATG
Q S L E Q Q L E L E K E K L G A H

CAGGCCACC TGCTGGAAA GATGGCCTG GCCAAGGCTC CATCTGTGC
Q A H L A G K M A L A K A P S V A

CTCAATGGAC AAGAGCTC TTCTGCATC GTAGCCACCA GTACTCAGGG
S M D K R A L A A S P P V L R

CAGTGTGCTC CCGGCTGGT CTGCTCCTCG GGAGCTCCA GACGGCGCC
A V C S R P G L L L G R L O T A A

TOTTTCAGT GCGGAGGCAC CTCTGGGAAA GCCATGGCAA TAGTCTCTC
C L Q C G G T S G E A K A I V P S

CCAGATCTT TCCACAAAT GGACTACTTC AAGTACCAAA ATATGGGACC
Q S S T T W T T S S T T I C D

insertion

premature termination

SCIENTIFIC BINDERY PRODUCTIONS CHICAGO 60605 MADE IN USA

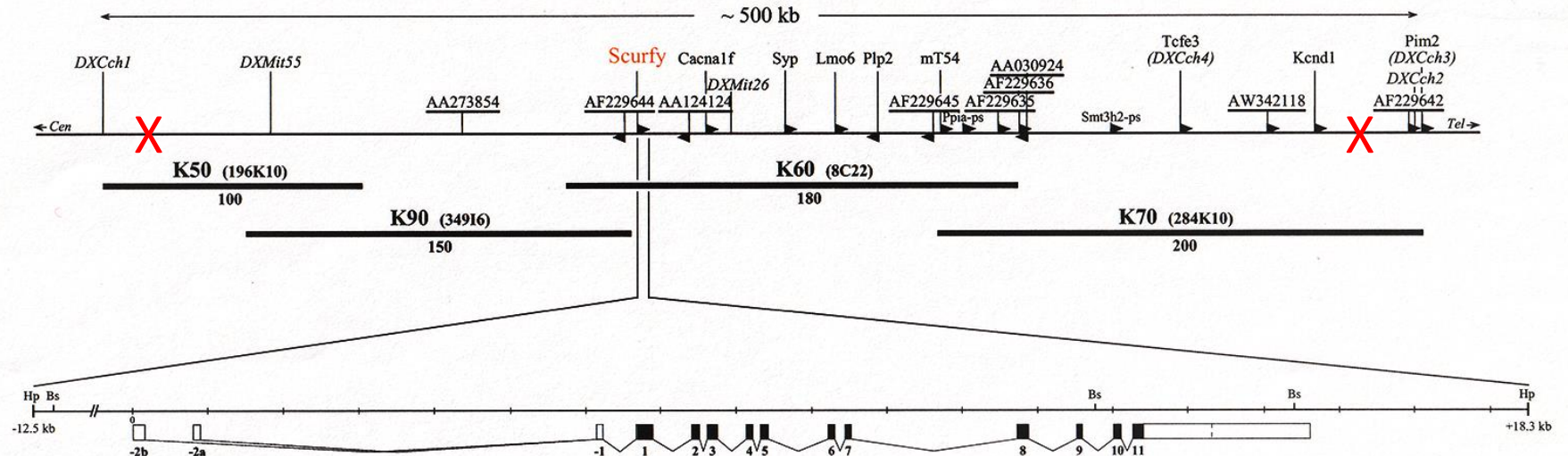
Work continued to Page 142

SIGNATURE *Kathryn Pfeiffer* DATE *4/9/98*

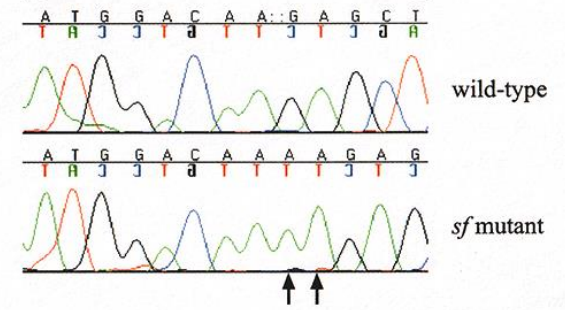
DISCLOSED TO AND UNDERSTOOD BY *NCS* DATE *01 May 98* WITNESS *ABO* DATE

9 April 1998

Molecular Genetic Basis of scurfy Phenotype

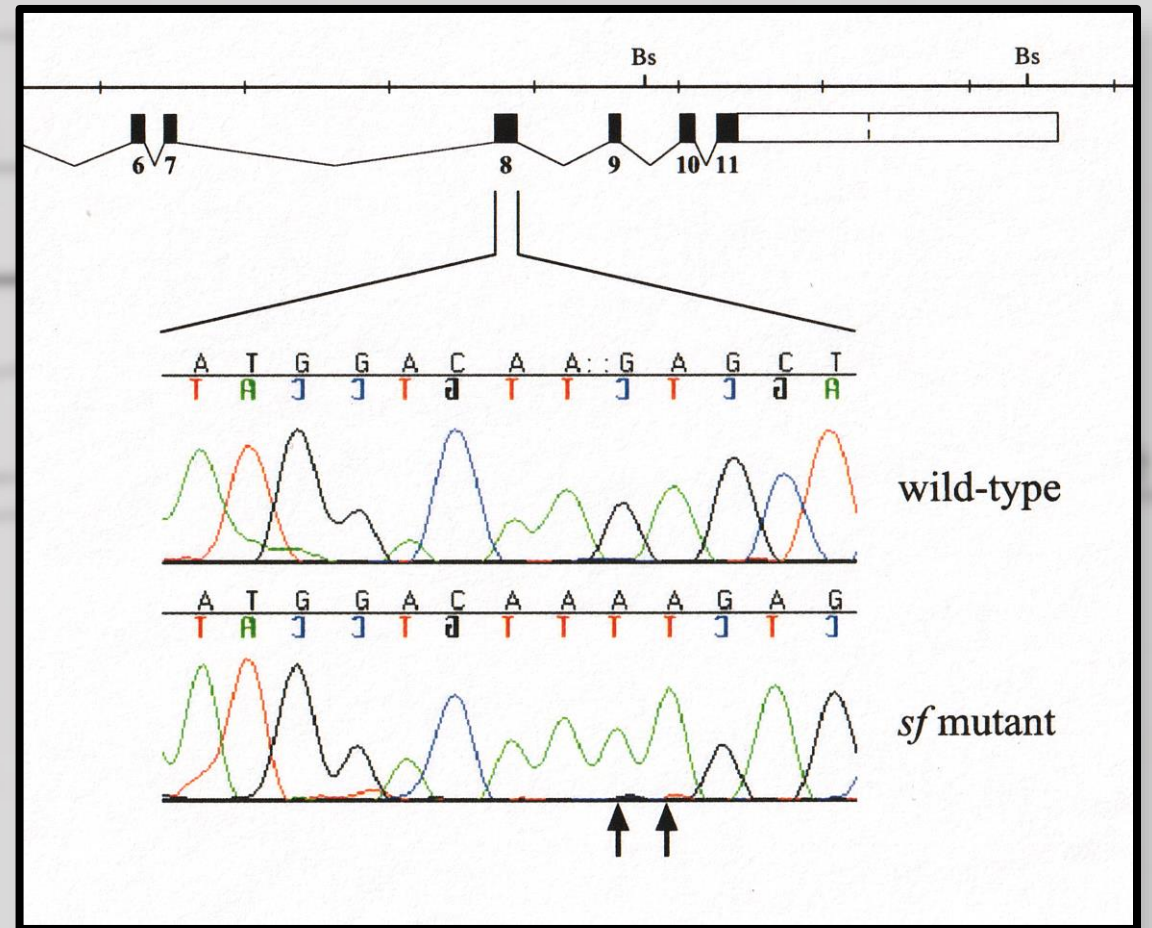


X Recombination breakpoints defining scurfy interval



2-base pair insertion in DNA-binding domain

Molecular Genetic Basis of scurfy Phenotype

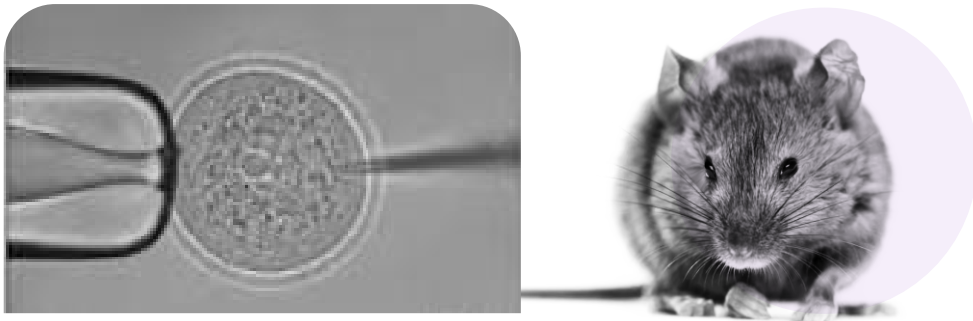
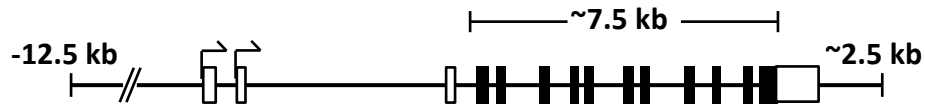


2-base pair insertion in DNA-binding domain

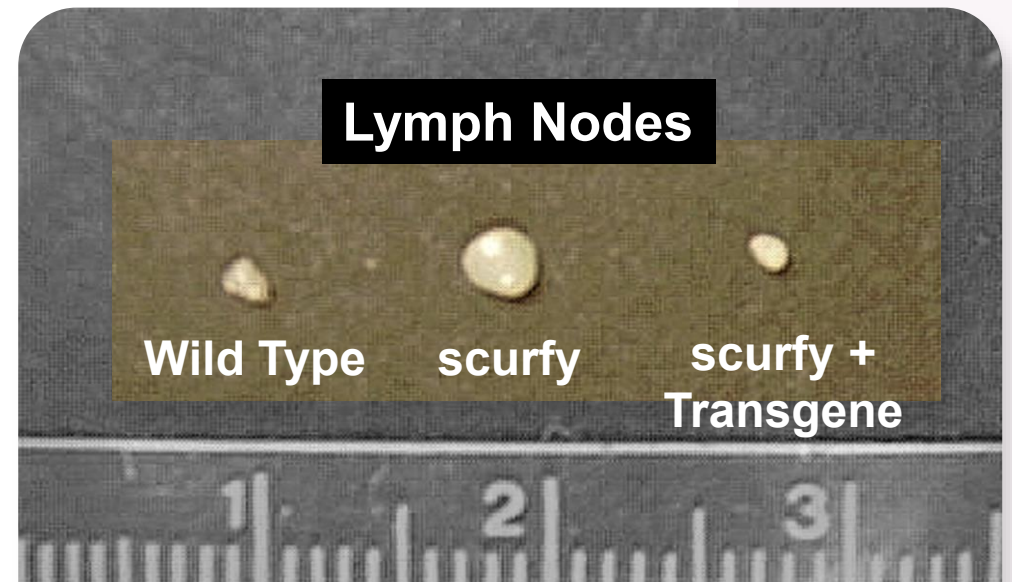
Confirmation that Foxp3 = scurfy

Transgene strategy

1. 30.8kb genomic fragment containing wild type Foxp3 gene rescues scurfy lethality



2. Effect of Foxp3 overexpression



FOXP3 = scurfy in Mice

Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse

Mary E. Brunkow¹, Eric W. Jeffery¹, Kathryn A. Hjerrild¹, Bryan Paeper¹, Lisa B. Clark¹, Sue-Ann Yasayko¹, J. Erby Wilkinson², David Galas³, Steven F. Ziegler⁴ & Fred Ramsdell¹

¹Celltech Chiroscience, Inc., Bothell, Washington, USA. ²Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA. ³Keck Graduate Institute for Applied Life Sciences, Claremont, California, USA. ⁴Virginia Mason Research Center, Seattle, Washington, USA. Correspondence should be addressed to M.E.B. (e-mail: marybrunkow@chiroscience.com).

FOXP3 = IPEX in Humans

Disruption of a new forkhead/winged-helix protein, scurfin, results in

Mary E. Brunkow¹, Eric
J. Erby Wilkinson², David

¹Celltech Chiroscience, Inc., Bothell, WA
²Applied Life Sciences, Claremont, CA
M.E.B. (e-mail: marybrunkow@

nature genetics
volume 27
january 2001

The immune dysregulation,
polyendocrinopathy, enteropathy,
X-linked syndrome (IPEX) is caused
by mutations of *FOXP3*

Craig L. Bennett^{1*}, Jacinda Christie^{2*},
Fred Ramsdell³, Mary E. Brunkow³, Polly
J. Ferguson⁴, Luke Whitesell⁵, Thaddeus
E. Kelly⁶, Frank T. Saulsbury⁶, Phillip F.
Chance¹ & Hans D. Ochs²

**These authors contributed equally to this work.*

fe

X-linked neonatal diabetes mellitus,
enteropathy and endocrinopathy
syndrome is the human equivalent of
mouse scurfy

Robert S. Wildin¹, Fred Ramsdell², Jane
Peake³, Francesca Faravelli⁴, Jean-Lau-
rent Casanova⁵, Neil Buist⁶, Ephrat Levy-
Lahad⁷, Massimo Mazzella⁸, Olivier
Goulet⁵, Lucia Perroni⁴, Franca Dagna
Bricarelli⁴, Geoffrey Byrne⁹, Mark
McEuen², Sean Proll², Mark Appleby² &
Mary E. Brunkow²

FOXP3 = IPEX in Humans

Mutations in human FOXP3 gene result in IPEX:

IMMUNE DYSREGULATION
POLYENDOCRINOPATHY
EENTEROPATHY
X-LINKED SYNDROME

Allelic series:

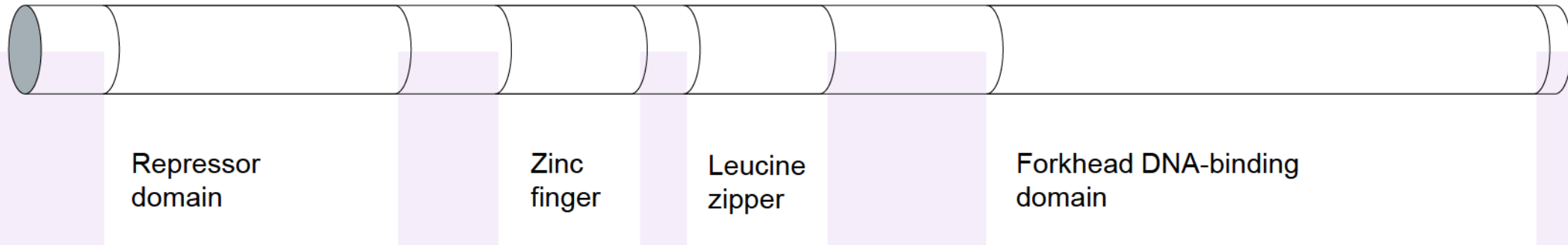
- Severity of disease depends on nature of the mutation (>170 mutations reported)

Significance

- Diagnosis
- Prognosis
- Structure/function analysis of FOXP3 protein

FOXP3 Encodes Transcription Factor

- Novel member of the forkhead/winged-helix gene family
- Highly conserved across all vertebrates
- Transcription repressor or activator, depending on interactors



| Drug Development from Gene Discovery

Indirect Human
Health Implications

*“Non-Druggable
Targets”*

Ex: FOXP3

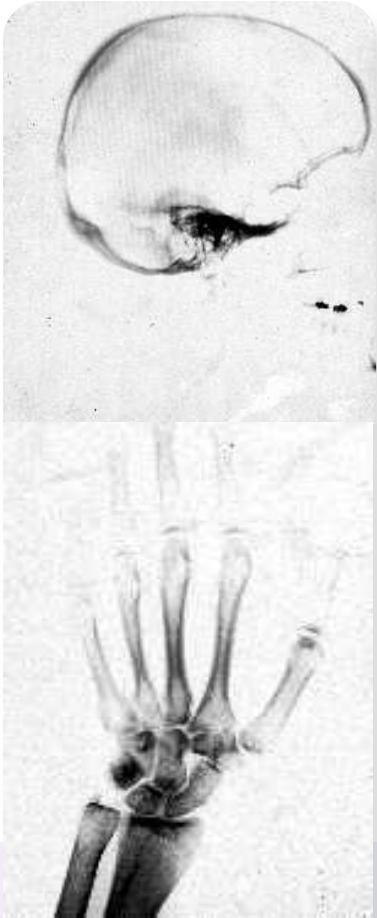
vs.

Direct Human
Health Implications

*“Druggable
Targets”*

Ex: SOST

Direct Clinical Application...



Normal



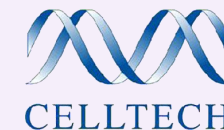
Sclerosteosis

Sclerosteosis (autosomal recessive)

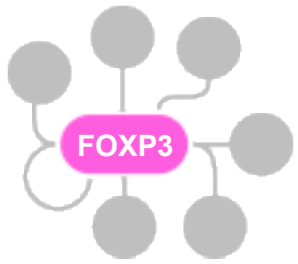
- **Excess deposition of skeletal bone:**
 - Throughout life
 - Extremely strong, deposited in normal manner
 - All bone types affected
 - Little other independent pathology
 - Carriers have intermediate bone density >> Dose-dependent!
- **Surrogate drug approach**
- **SOST:** Member of a family of **secreted BMP antagonists**

Brunkow et al AJHG 2001 | Winkler et al EMBO 2003

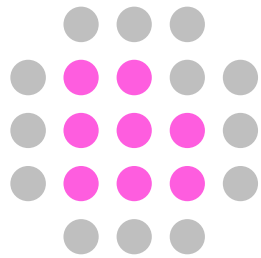
Blocking antibody is now on the market! (Romosozumab/Evenity)



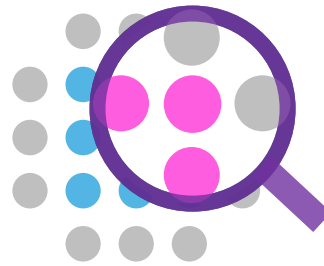
FOXP3 / Scurfin: Post-Cloning



Gene regulatory networks
New drug targets?



Limited Expression
(5-10% of CD4+ cells
are also CD25+)



Treg-specific Marker
New hook into Treg
cell biology



FOXP3 programs Treg identity
Master regulator of
Treg cells



Treg-associated Therapies
Autoimmune disease
Organ transplantation
Cancer

Acknowledgements



David Galas

Steve Ziegler

Fred Ramsdell

Eric Jeffrey

Kathryn Hjerrild

Bryan Paeper

Roli Khattri

Mark Appleby

Lisa Clark

Stephanie Corpening

Tom Cox

Diane Isonaka

Brian Kovacevich

Mark McEuen

Marty Mortrud

Pat Moss

John Mulligan

Sean Proll

Sequencing Core

Don Walker

Sue-Ann Yasayko

J. Erby Wilkinson

Virginia Godfrey

Dabney Johnson

Patrick Blair

Sharon Witonsky

Hans Ochs

Craig Bennett

Phillip Chance

Robert Wildin



Jim Heath

Lee Hood

22 October 2025

