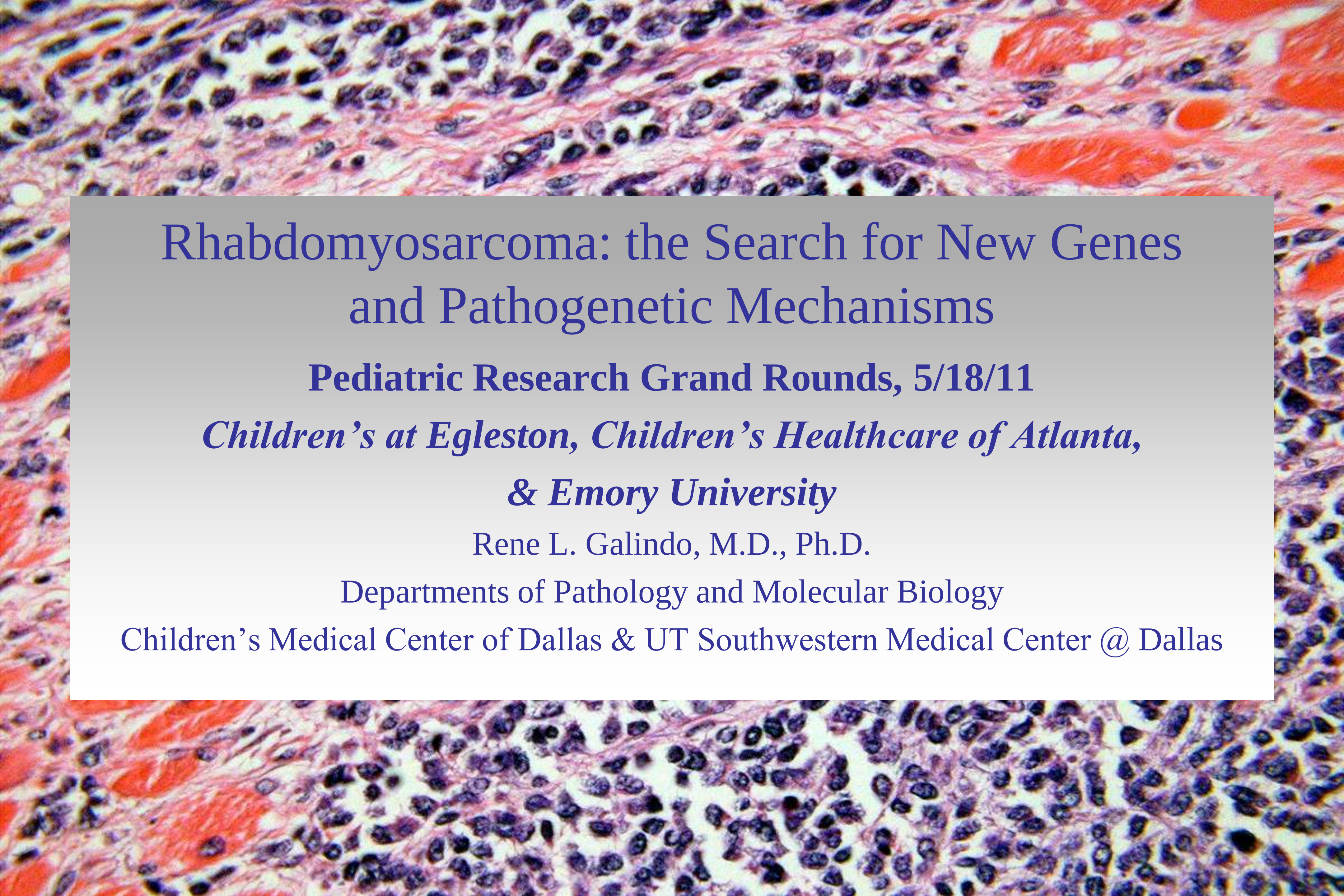


Rhabdomyosarcoma: the Search for New Genes and Pathogenetic Mechanisms

Pediatric Research Grand Rounds, 5/18/11

Children's at Egleston, Children's Healthcare of Atlanta, & Emory University

Rene L. Galindo, M.D., Ph.D.

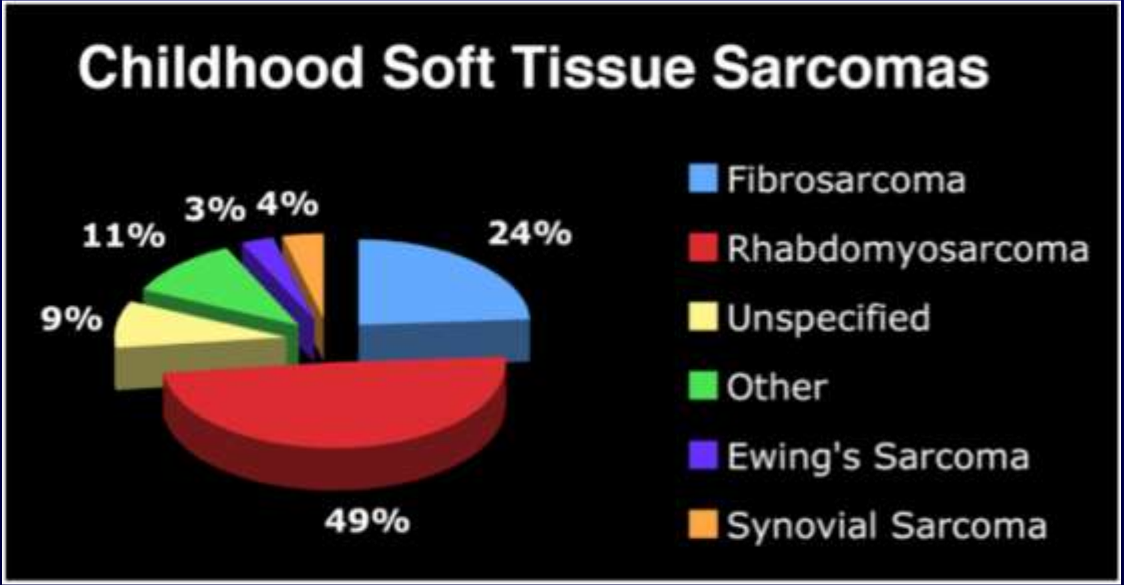
Departments of Pathology and Molecular Biology

Children's Medical Center of Dallas & UT Southwestern Medical Center @ Dallas

Childhood Development and Disease

- "Children are not just little adults"- includes childhood cancer
- Children do not suffer from the typical tumors of adulthood (*e.g.*, epithelial carcinomas)
- Instead, pediatric solid tumors are typically of connective tissue (sarcomas) or neuroectodermal origin
- Why do sarcomas prefer childhood tissue?

Genetics of Childhood Sarcomas

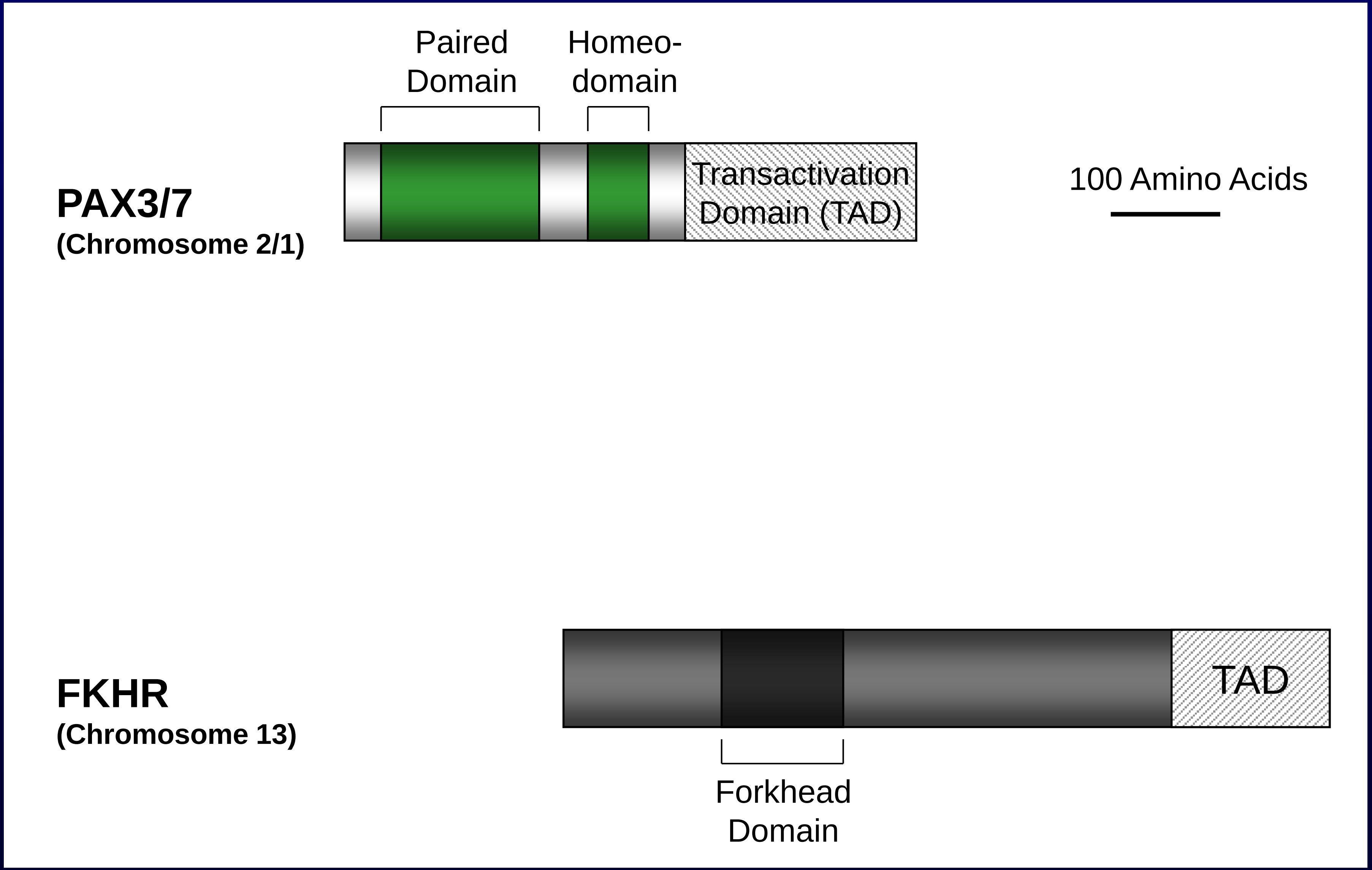


Adapted from SEERS program data, NCI

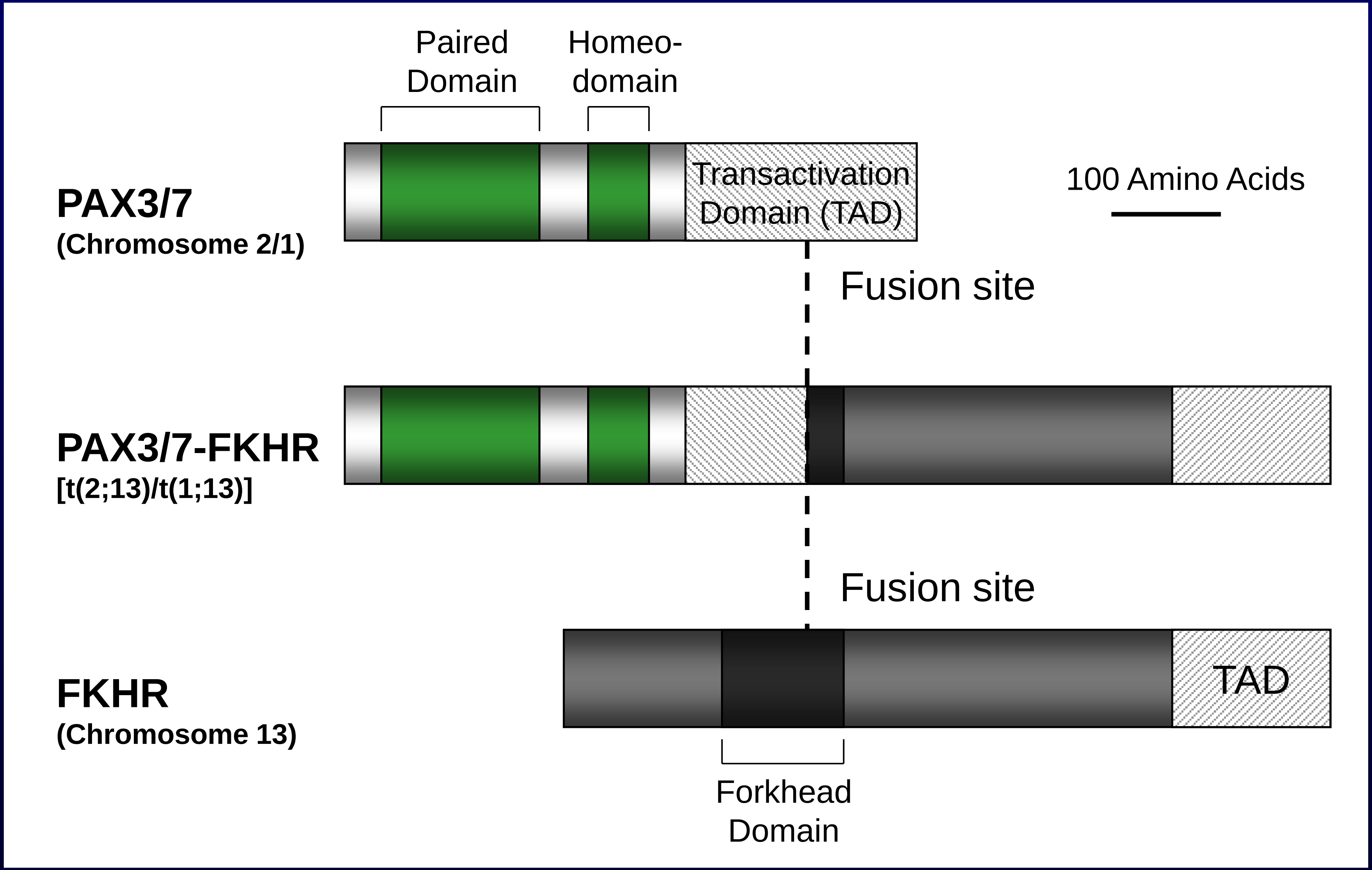
- Childhood sarcomas are genetically distinct diseases
  - Rhabdomyosarcoma (RMS)
    - t(2;13), t(1;13); PAX-FKHR
  - Ewing's Sarcoma
    - t(11;12); EWS-FLI1
  - Synovial Sarcoma
    - t(X;18); SYT-SSX
- Lack of true/efficient animal models
  - Toxicity of fusion oncoproteins
  - Unknown cell/tissue of origin
- RMS is a tumor of skeletal muscle-type histology, and most common
- PAX-FKHR RMS is notoriously aggressive

Rhabdomyosarcoma and PAX-FKHR

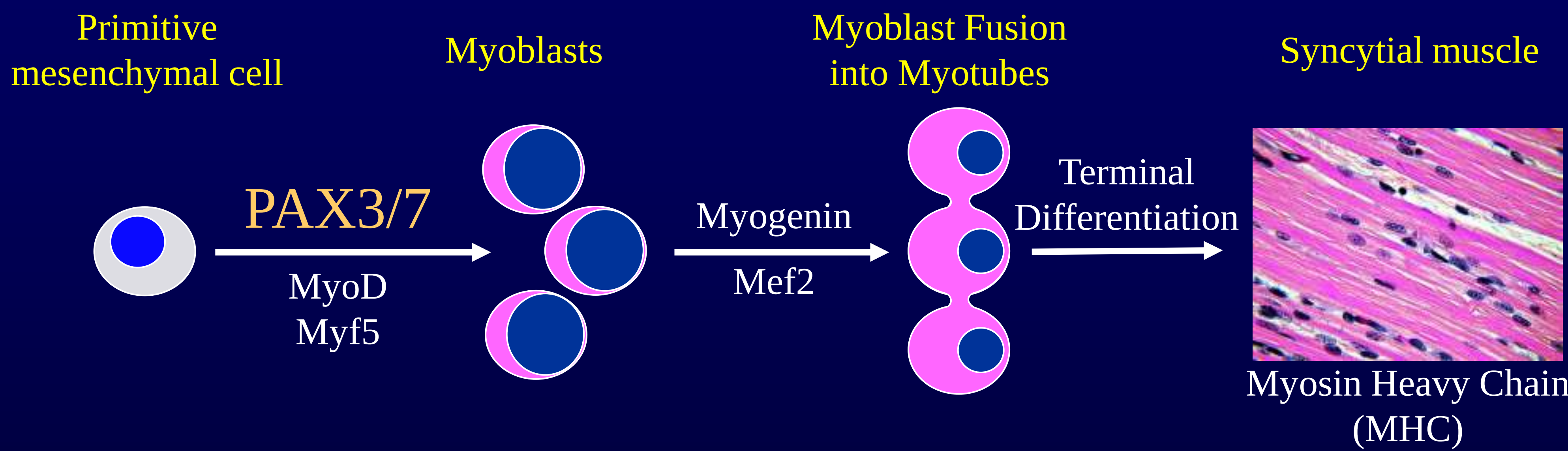
The PAX-FKHR Protein Fusion



The PAX-FKHR Protein Fusion

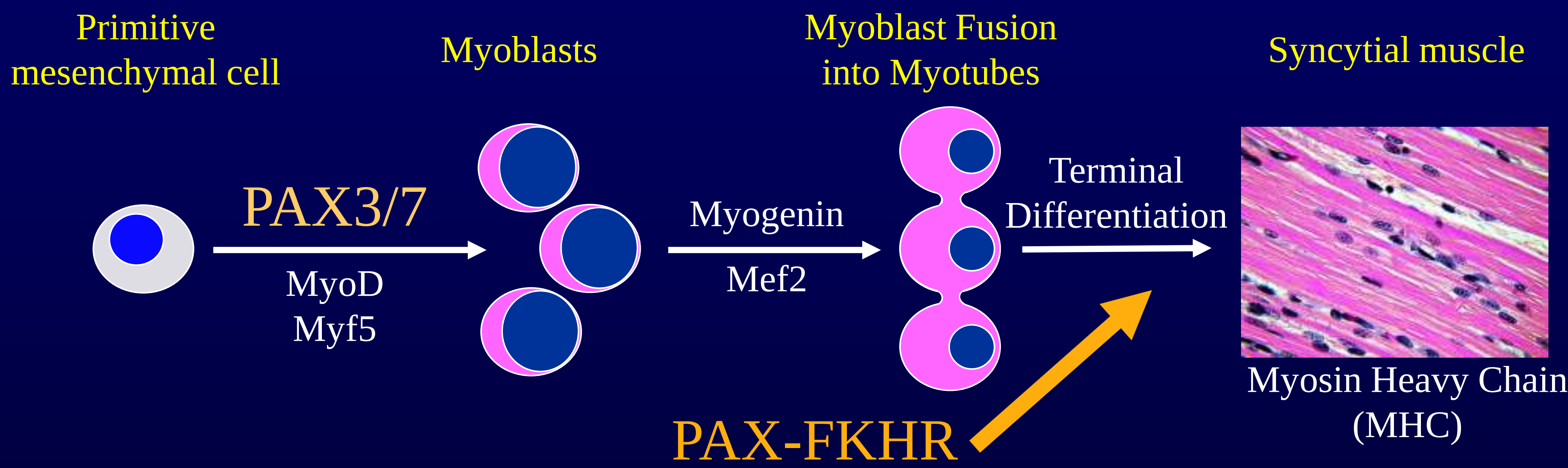


Myogenesis: Myoblasts, Cell-Cell Fusion, and then Muscle



- Little is known regarding PAX3/7 gene targets and cofactors
- Gene redundancy in mammals
- The underpinnings of PAX-FKHR pathogenesis remain poorly understood

Myogenesis: Myoblasts, Cell-Cell Fusion, and then Muscle



- Keller et al., *Genes Dev*, 2004- PAX3-FKHR expressed in differentiating muscle caused RMS tumorigenesis
- However, suboptimal tumor incidence and latency for *in vivo* profiling of PAX-FKHR cell biology and pathogenesis

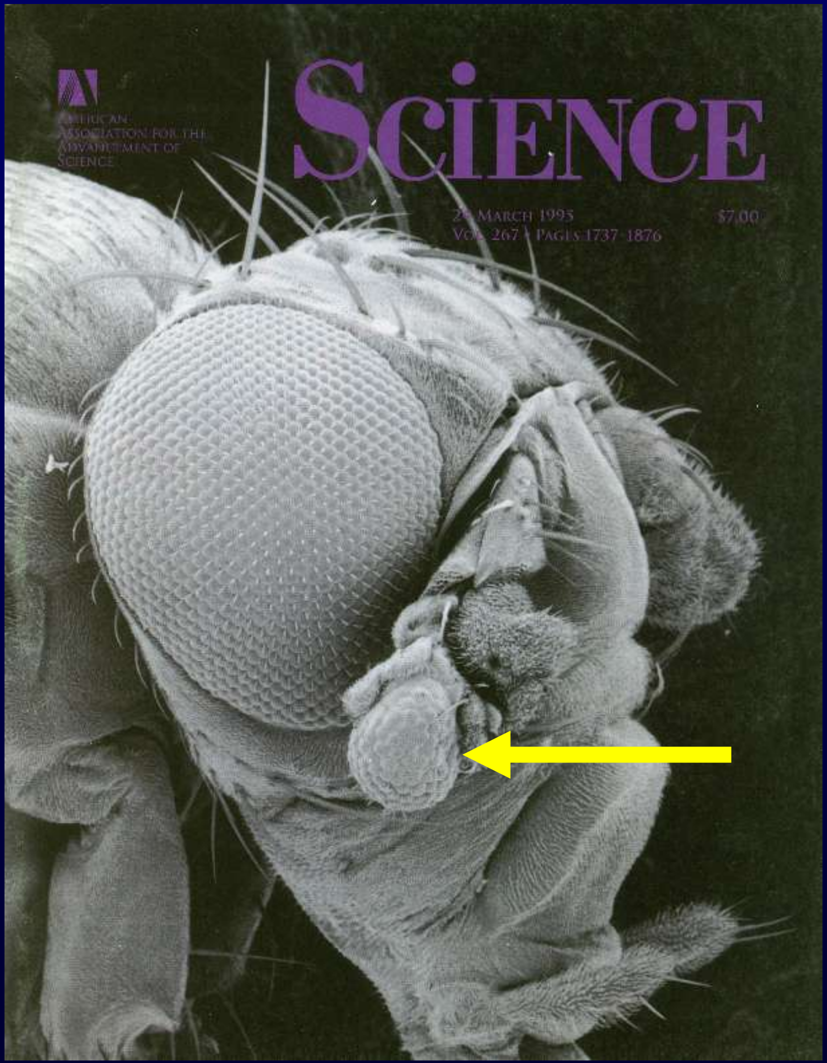
A *Drosophila* PAX-FKHR Model System

A *Drosophila* PAX-FKHR Model System

Drosophila melanogaster
(http://flymove.uni-muenster.de)

- Entire, intact organisms can be examined by conventional microscopy
- Fluorescent protein reporters
- Conditional expression of transgenes
- Unbiased forward genetic screens
- Rich conservation of mammalian genes-particularly PAX

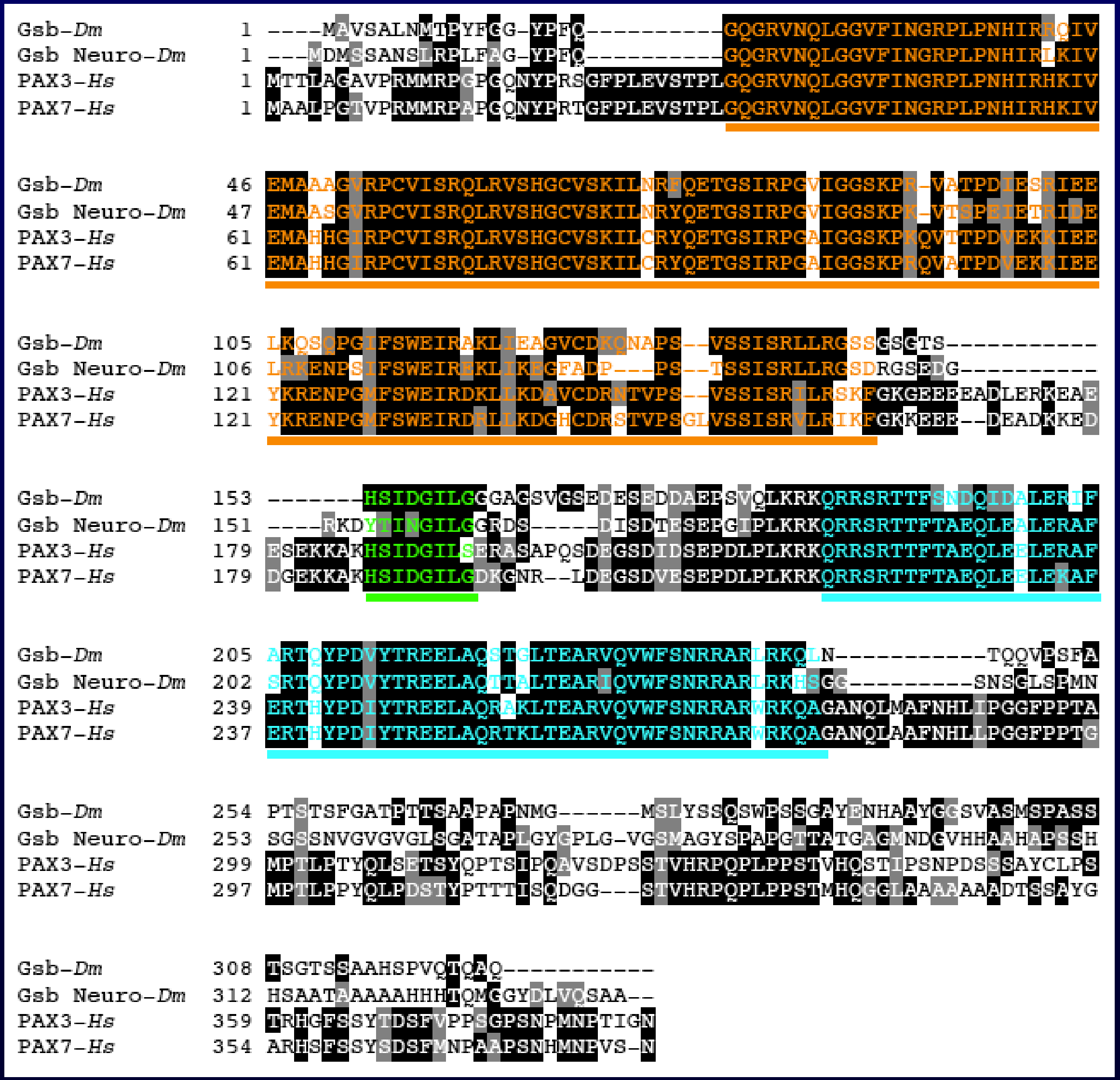
Human and Fly PAX



Halder *et al.*, 1995

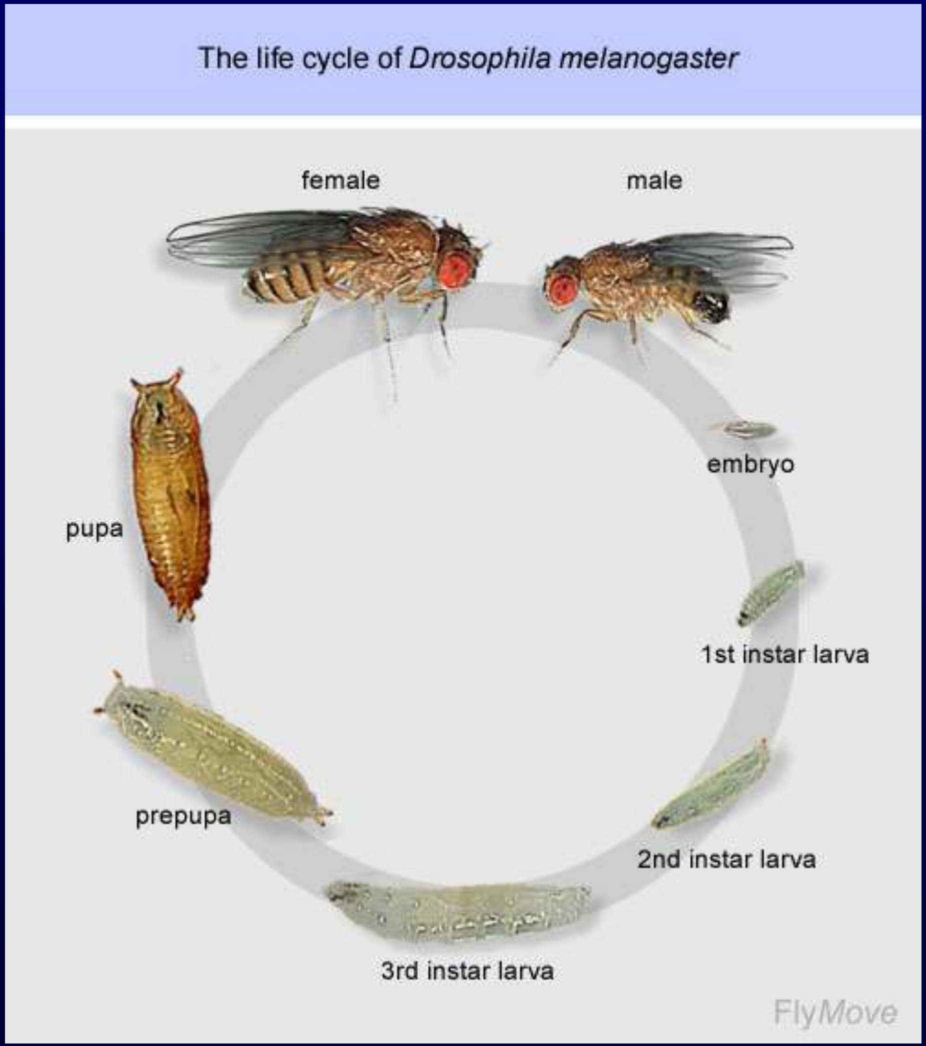
- PAX6- master regulator of eye development in flies and mammals
- PAX6 misexpression in flies causes ectopic eyes
- True for BOTH fly and mammalian PAX6
- Why not PAX3/7-FKHR?

Human and Fly Pax3/7



- *Drosophila* and mammalian PAX3/7 are structurally and functionally conserved
- PAX3- *e.g.*, Xue and Noll, *Development*, 1996
- Both mammalian and fly PAX3/7 participate in muscle biology
- Again, why not PAX-FKHR?

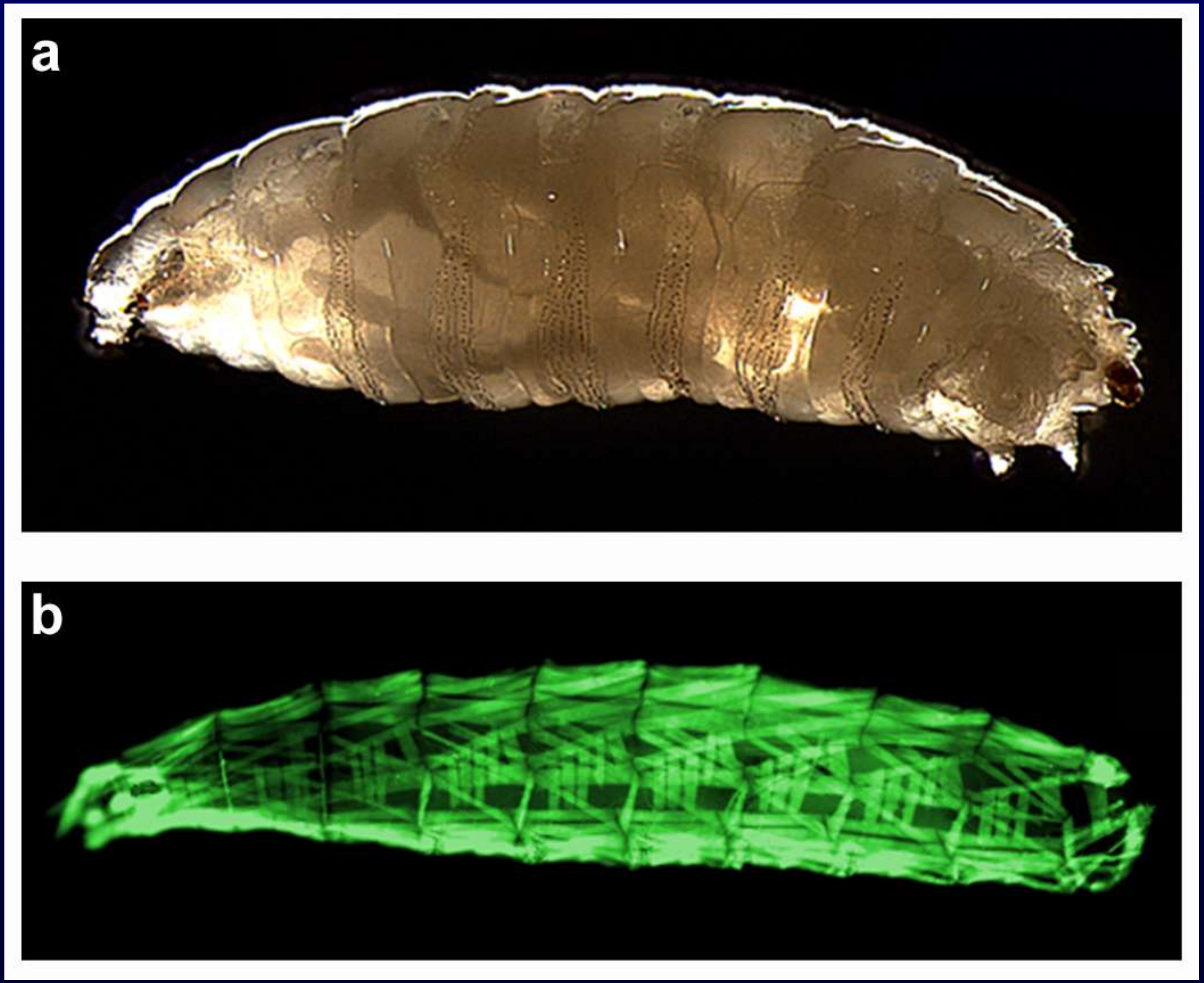
PAX-FKHR in *Drosophila* Muscle



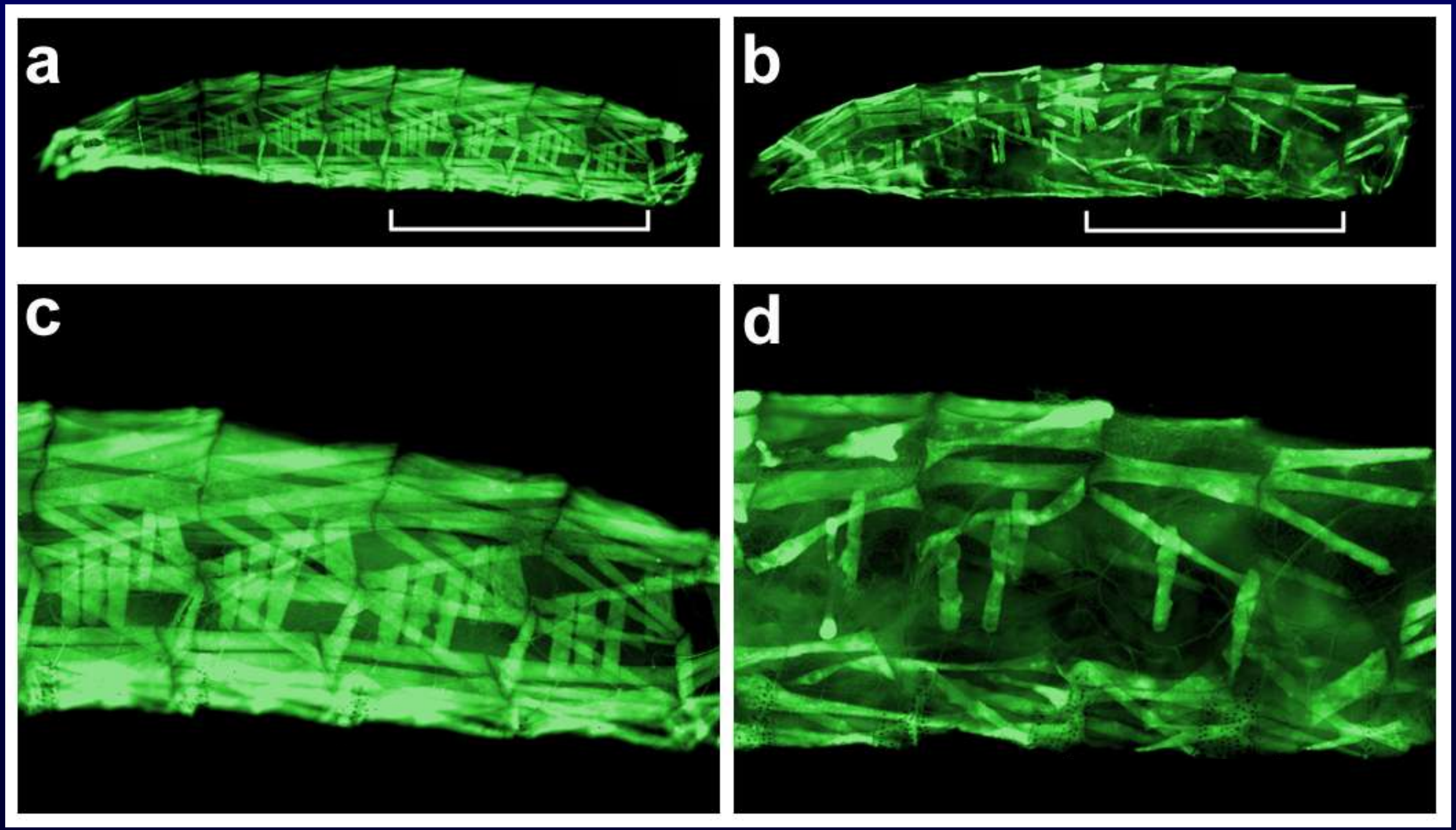
Weigmann et al., 2003, *Trends Genet.*
http://flymove.uni-muenster.de

- *Myosin heavy chain (MHC)* regulatory elements to direct *PAX-FKHR* expression in differentiated syncytial muscle
- Expression is predominantly post-embryonic
- Targets larval juvenile muscle undergoing physiologic growth

Real-Time Examination of *Drosophila* Muscle



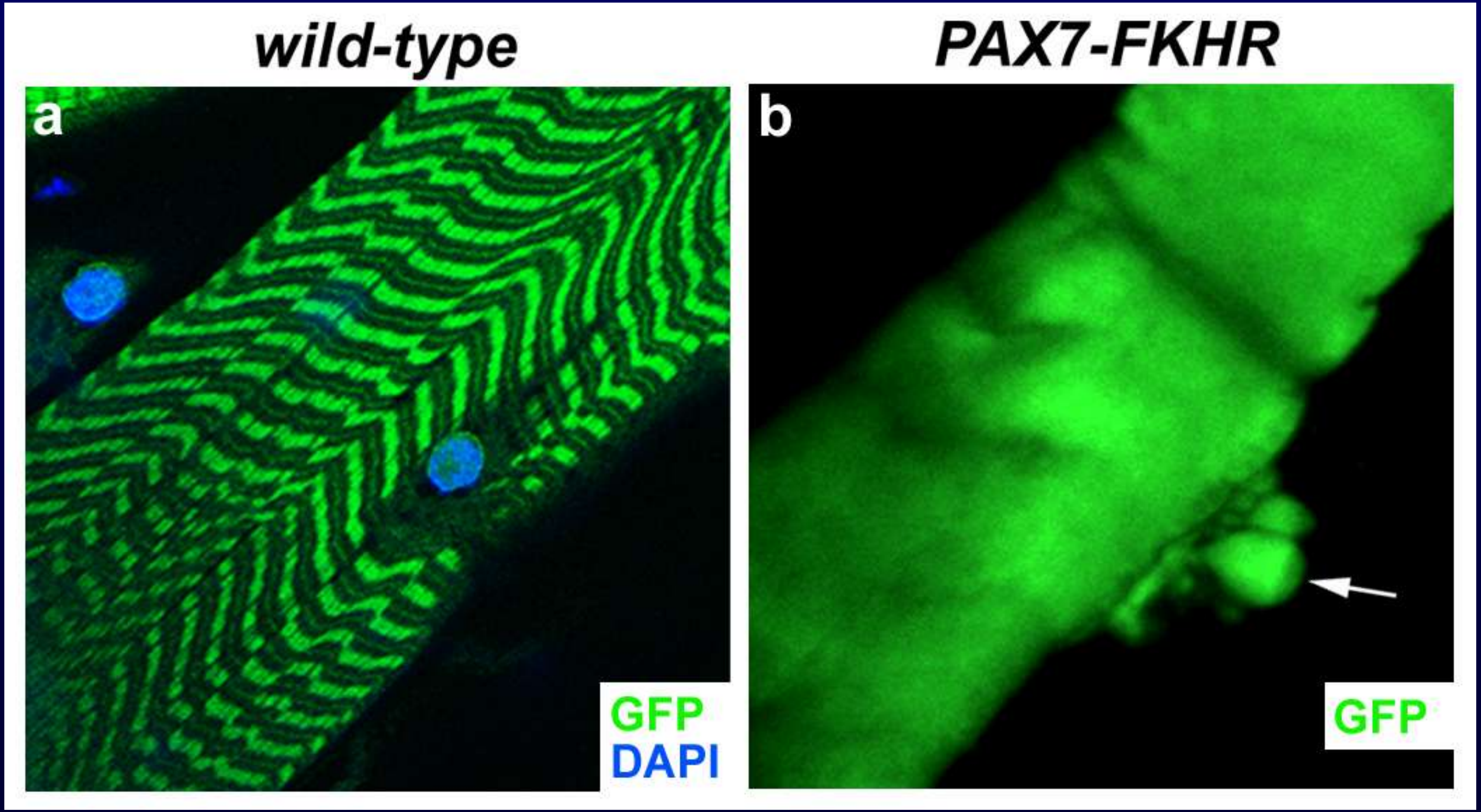
PAX-FKHR Compromises Muscle Patterning



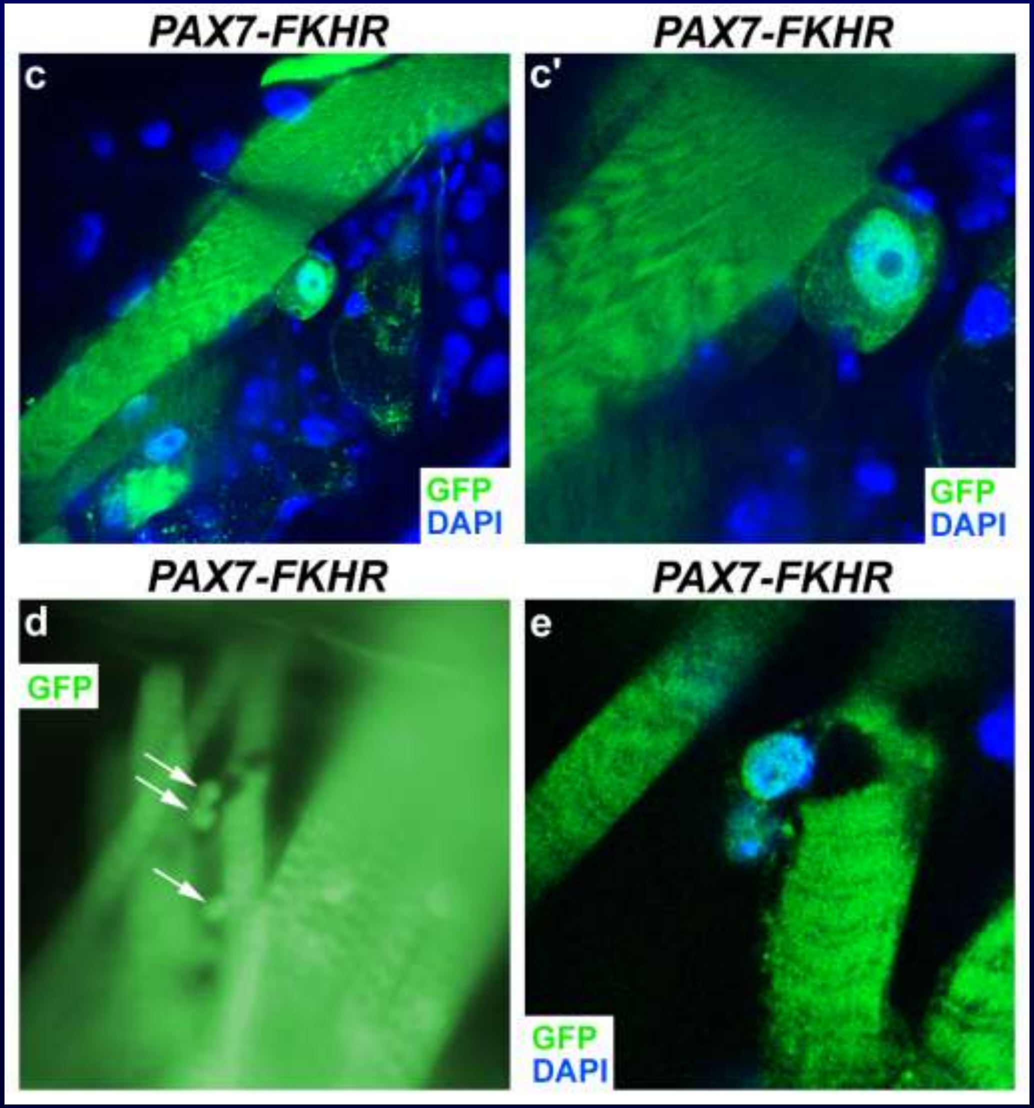
MHC>>GFP

MHC>>GFP, PAX-FKHR

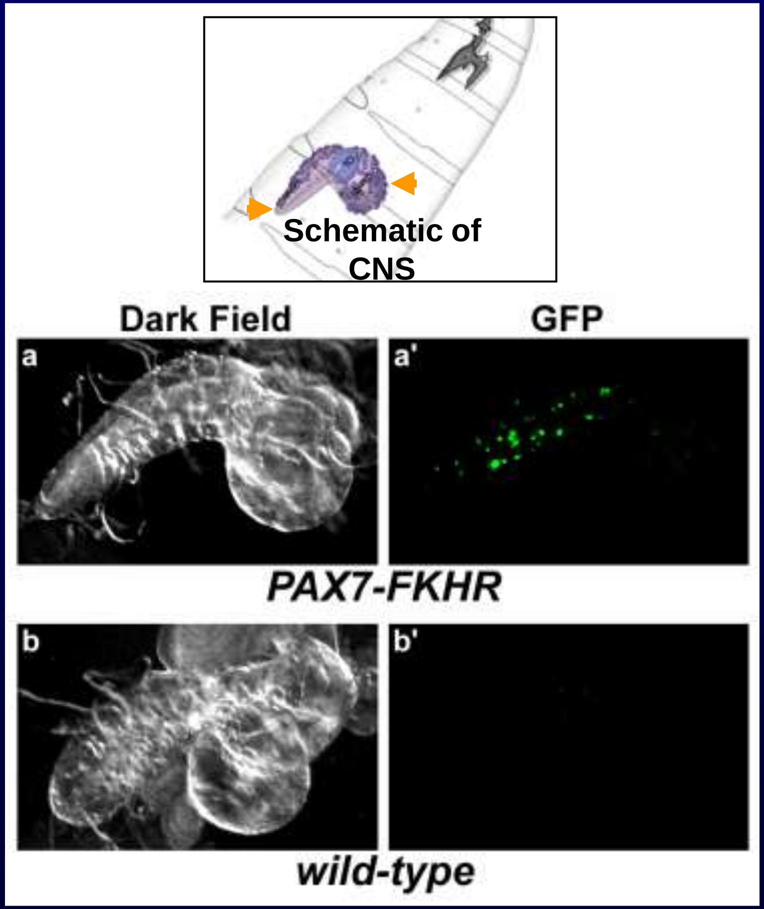
PAX-FKHR Muscle Demonstrates Fusion Defects

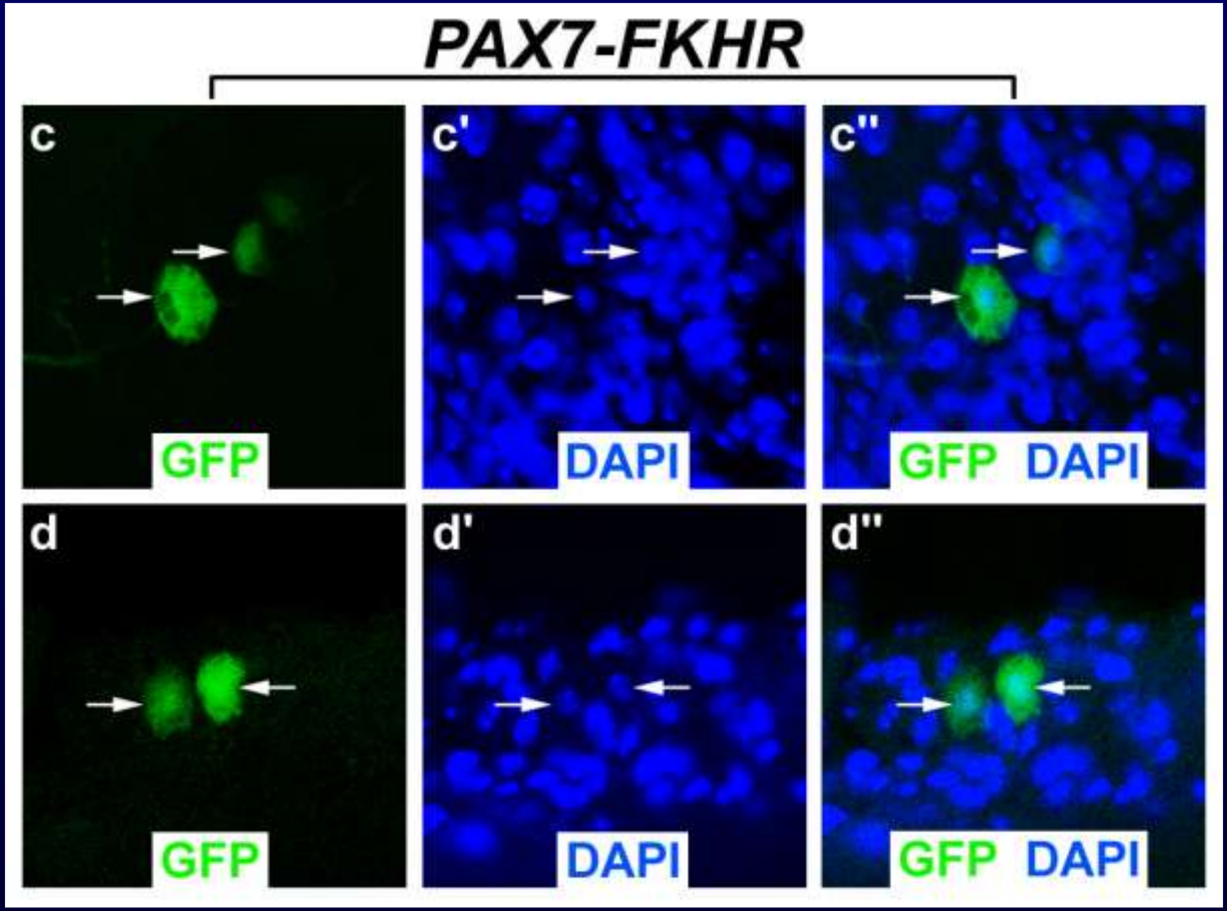


PAX-FKHR Muscle Demonstrates Fusion Defects



PAX-FKHR Myogenic Tissue in the Larval Central Nervous System





"Ok, but it is screenable?"

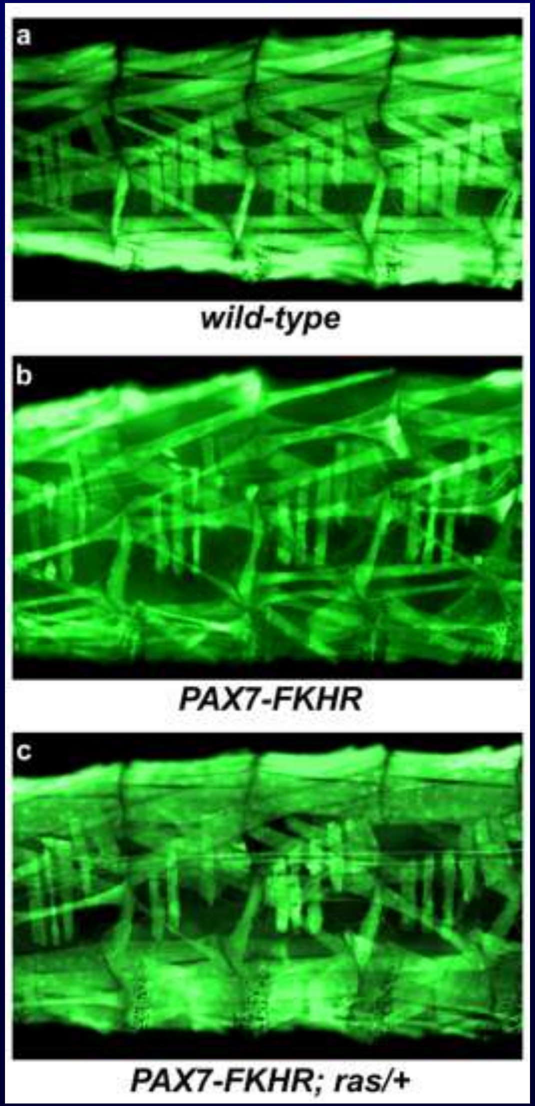
Eric Olson, circa 2007

An Invertebrate Genetic Approach to the Study of PAX-FKHR

Drosophila melanogaster
(http://flymove.uni-muenster.de)

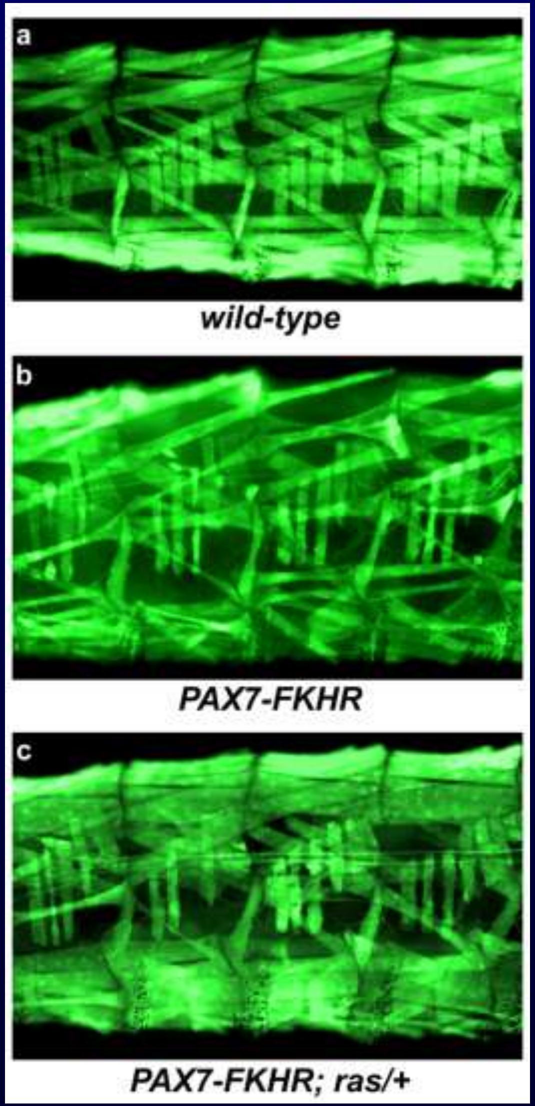
- Entire, intact organisms can be examined by conventional microscopy
- Fluorescent protein reporters
- Conditional expression of transgenes
- Unbiased forward genetic screens
- Rich conservation of mammalian genes- particularly PAX

Ras Loss-of-Function Suppresses *PAX-FKHR* Activity and Rescues Lethality



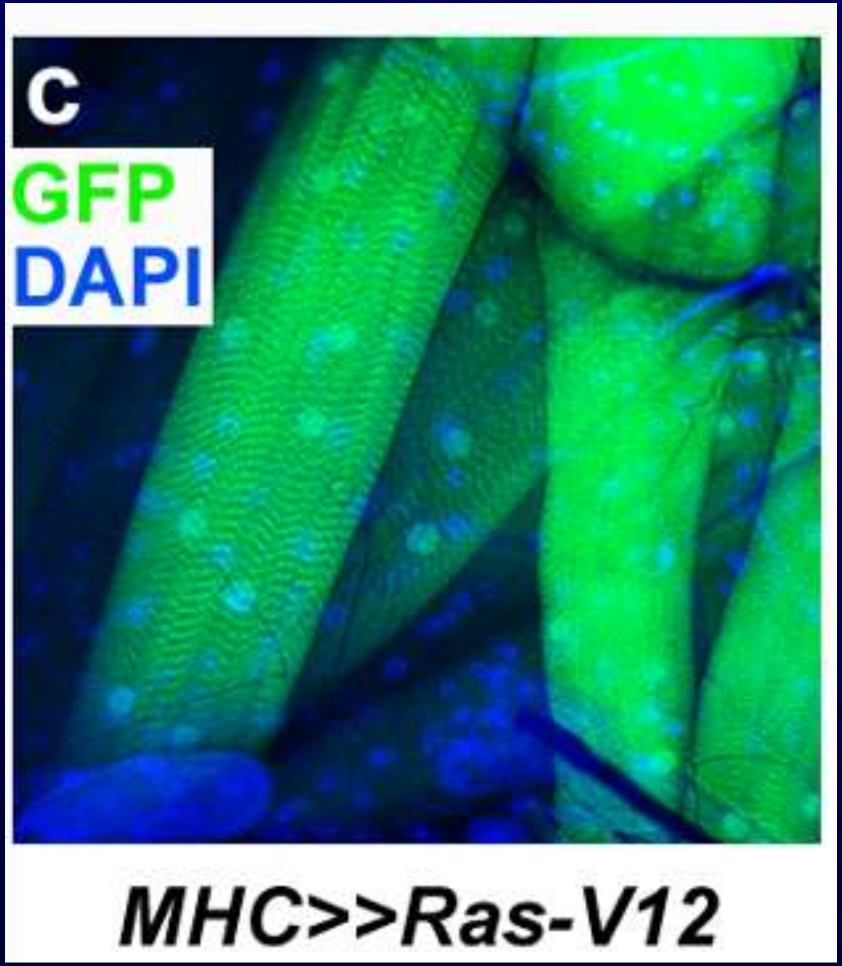
- ✓ Tested loss-of-function *ras* mutation for PAX-FKHR suppression
- ✓ Dominantly suppressed PAX-FKHR pathogenicity in muscle
- ✓ Rescues PAX-FKHR larval lethality to adult viability

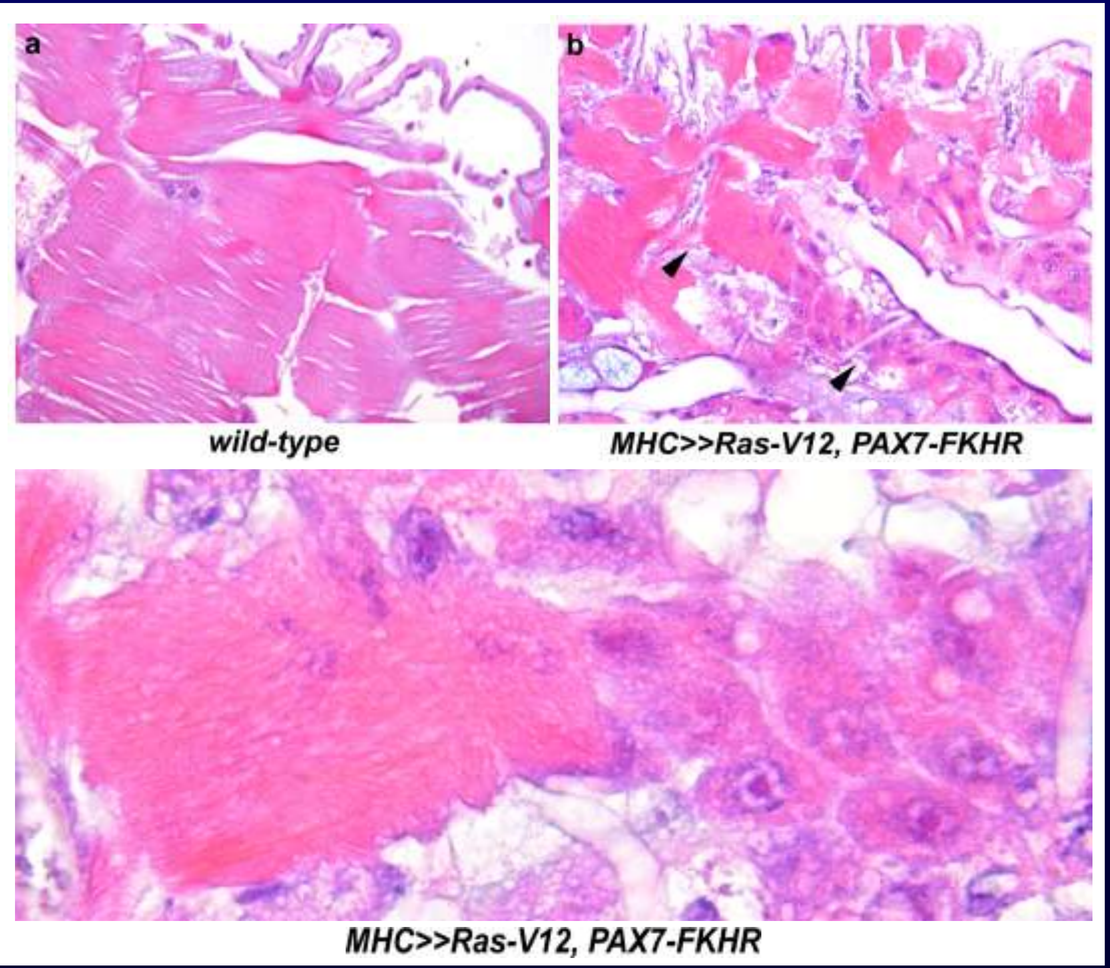
Ras Loss-of-Function Suppresses *PAX-FKHR* Activity and Rescues Lethality



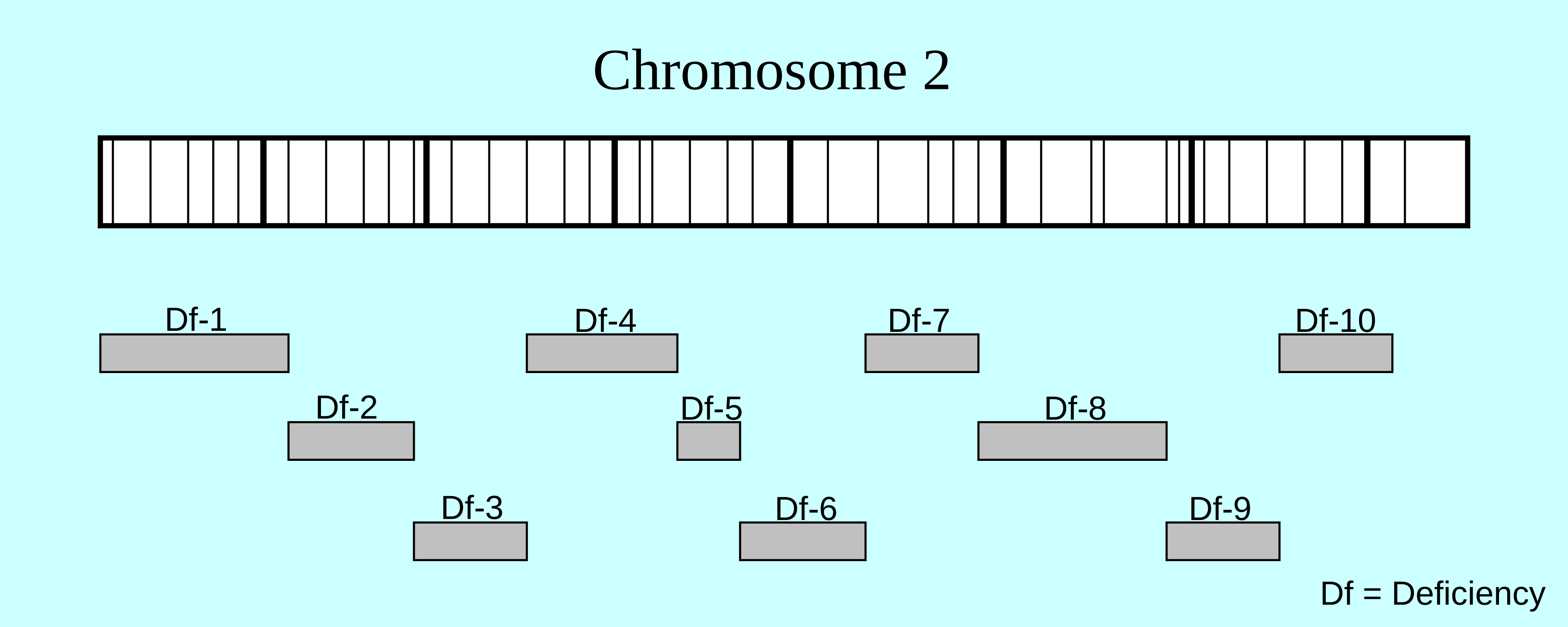
- ✓ Tested loss-of-function *ras* mutation for PAX-FKHR suppression
- ✓ Dominantly suppressed PAX-FKHR pathogenicity in muscle
- ✓ Rescues PAX-FKHR larval lethality to adult viability

Ras^{V12} Gain-of-Function and *PAX-FKHR* muscle

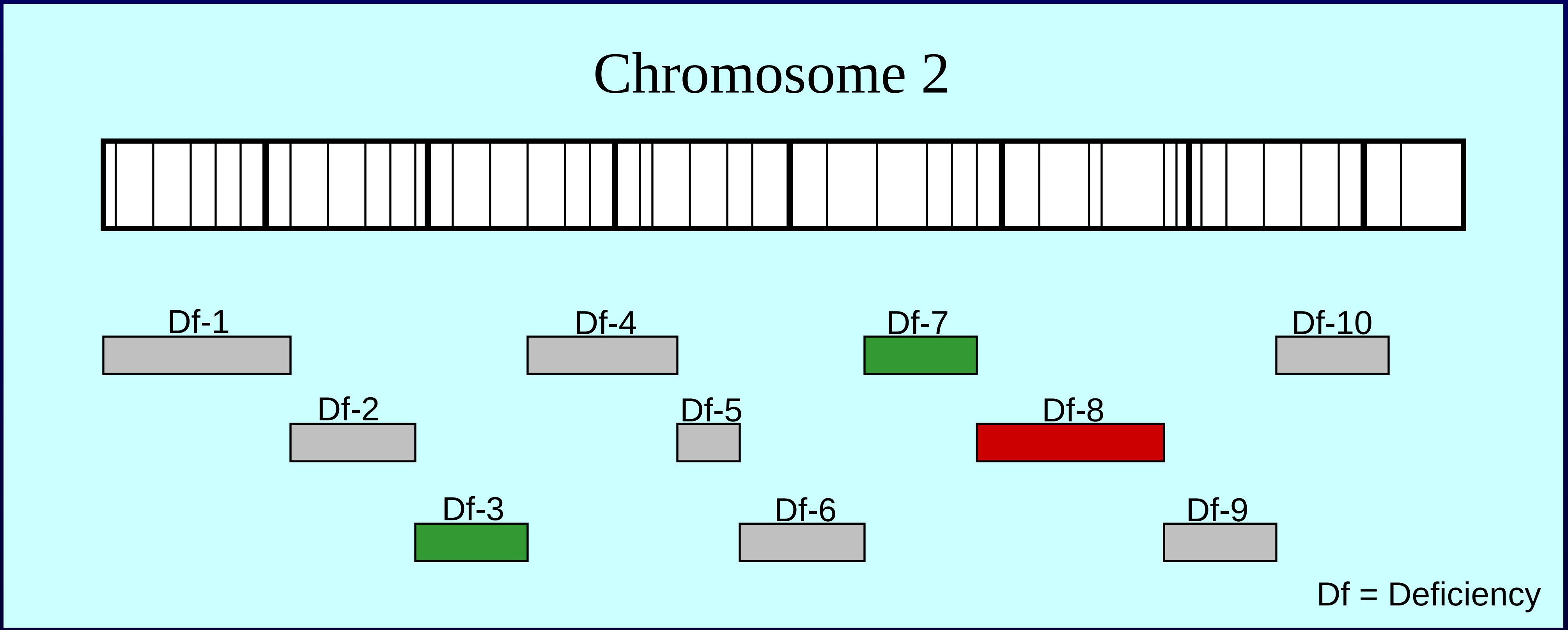




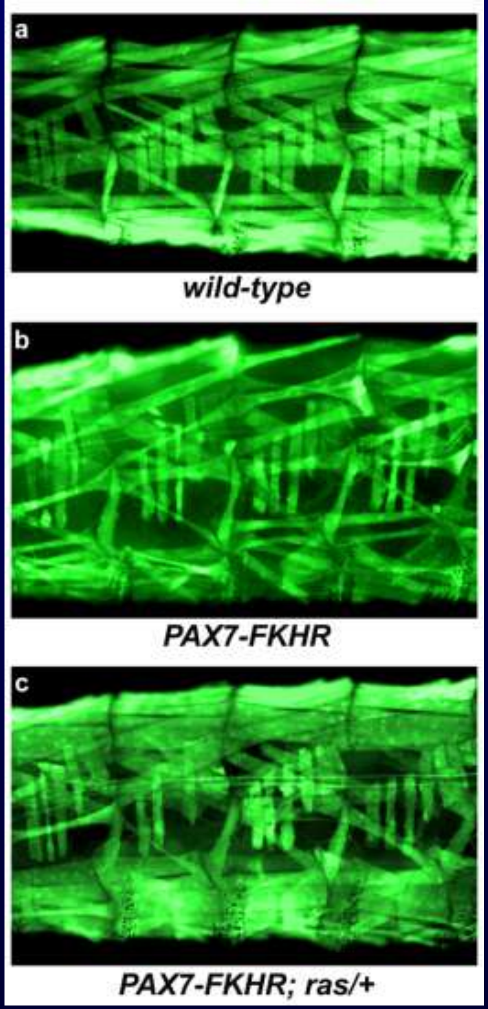
Deficiency Screening



Deficiency Screening



PAX-FKHR Deficiency Screening



Deficiency Line	# Flies Examined	"Expression-OFF" *Df* *MHC>>PAX7-FKHR*	"Expression-ON" *Df* *MHC>>PAX7-FKHR*
"Expected" (Mendelian Ratios)	-	50	50
None (control 1)	75	71	29
None (control 2)	58	72	28
Df(2L)pr-A16	32	66	34
Df(2L)C'	42	69	31
Df(2L)BSC16	53	64	36
Df(2L)E110	49	47	53
Df(2L)BSC36	58	48	52
Df(2L)ast2	49	88	12
Df(2L)BSC30	37	86	14

Non-modifier (yellow)
Suppressor (green)
Enhancer (red)

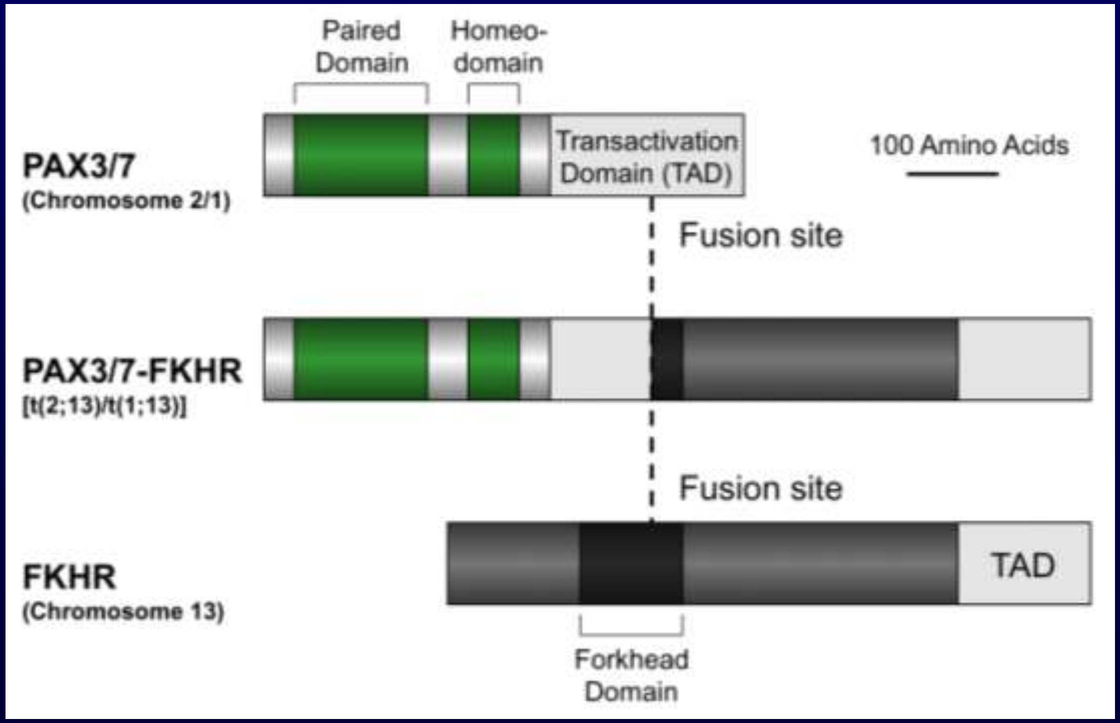
PAX-FKHR Screening and Results

95F7;96A17-18	2363	0	194	194	0%
95D7-D11;95F15	4432	0	225	225	0%
61A;61D3	2577	0	115	115	0%
75A6-7;75C1-2	2608	0	217	217	0%
64C;65C	3096	0	53	53	0%
75F10-11;76A1-5	6754	0	77	77	0%
56F9-17;57D11-12	2467	1	75	76	1%
85A2;85C1-2	1962	3	189	192	2%
70C1-2;70D4-5	3124	1	50	51	2%
49C1-4;50C23-D2	442	2	88	90	2%
65A2;65E1	4393	3	88	91	3%
60E2-3;60E11-12	2471	5	140	145	3%
71F1-4;72D1-10	190	5	124	129	4%
63F6-7;64C13-15	3686	4	97	101	4%
94A3-4;94D1-4	2586	3	71	74	4%
49A4-13;49E7-F1	754	6	128	134	4%
93B6-7;93D2	3340	5	98	103	5%
57D2-8;58D1	5246	8	140	148	5%
88E7-13;89A1	383	13	175	188	7%
54B2;54B17	9595	5	66	71	7%
81F3-6;82F5-7	1518	9	108	117	8%
60C8;60E8	9069	30	332	362	8%
53D9-E1;54B5-10	7445	12	112	124	10%
54C1-4;54C1-4	5574	20	180	200	10%
85D8-12;85E7-F1	1931	19	170	189	10%
97E3;98A5	823	18	152	170	11%
41A;41A	739	22	167	189	12%
60F1;60F5	4961	11	80	91	12%
66B8-9;66C9-10	1541	19	129	148	13%
63E6-9;64A8-9	463	42	276	318	13%
84A5;84D9	1842	18	106	124	15%
82F8-10;83A1-3	5694	34	196	230	15%
62A10-B1;62D2-5	600	14	80	94	15%
96F1;96F10	9500	17	93	110	15%
77B-C;77F-78A	3127	35	190	225	16%
59D5-10;60B3-8	1682	18	94	112	16%
44D1-4;44F12	201	38	196	234	16%
56F12-14;57A4	6609	14	71	85	16%
99B5-6;99F1	3547	51	254	305	17%
95D8;95E5	7676	22	109	131	17%
99D3;3Rt	3546	41	197	238	17%
???	447	17	81	98	17%
50E4;50F6	7876	29	136	165	18%
96C9;96E2	7681	32	150	182	18%
77F3;78C8-9	4429	83	387	470	18%
67E3-7;68A2-6	6471	21	96	117	18%
48A3-4;48C6-8	1145	22	97	119	18%
96F1;97B1	5601	28	123	151	19%
84A1-2;84B1-2	1884	26	114	140	19%
83B7-C1;83C6-D1	7443	32	140	172	19%
51D3-8;52F5-9	3518	27	118	145	19%
78D5;79A2	8101	93	390	483	19%
42A1-2;42E6-F1	1007	28	116	144	19%
83E1-2;84A5	2393	19	78	97	20%
	w1118	98	390	488	20%
71F1-4;72D1-10	3640	72	278	350	21%
97F1-2;98A	9529	46	176	222	21%
62A2;62A7	7566	103	389	492	21%
54B17-C4;54C1-4	5680	34	126	160	21%
77E2-4;78A2-4	5878	44	163	207	21%
	w1118	75	272	347	22%
73A3;74F	2998	39	134	173	23%
96E2;96E6	7682	37	160	164	23%
98E3;99A6-8	430	63	210	273	23%
95A2-4;95A8-B1	9497	43	141	184	23%
94E9;94E13	7990	37	118	155	24%
95C12;95D8	7675	52	155	207	25%
	w1118	74	218	292	25%
54B1-2;54B7-10	7414	47	133	180	26%
60C5-6;60D9-10	2604	39	109	148	26%
66A17-20;66C1-5	5877	23	62	85	27%
68C8-11;69B4-5	2612	47	126	173	27%
86C1;87B1-5	3128	38	99	137	28%
77A1;77D1	2052	58	150	208	28%
77A1;77D1	8082	35	90	125	28%
75F2;76A1	757	31	79	110	28%
69A4-5;69D4-6	5492	43	107	150	29%
58D1-2;59A	282	40	92	132	30%
83B4;83B6	8103	50	104	154	32%
55A;55F	1547	71	147	218	33%
95B1;95D1	7992	53	109	162	33%
69F6-70A1;70A1-2	6457	36	74	110	33%
98E3;99A6-8	430	38	78	116	33%
45F6;46B12	9410	43	86	129	33%
59A1-3;59D1-4	3909	43	85	128	34%
97A;98A1-2	1910	90	176	266	34%
65F3;66B10	1420	41	79	120	34%
56C4;56D6-10	6866	48	88	136	35%
72C1-D1;73A3-4	2993	58	106	164	35%
98E3;99A6-8	430	39	70	109	36%
62B4-7;62D5-E5	2400	51	89	140	36%
46A;46C	1743	50	86	136	37%
54D1-2;54E5-7	6779	58	99	157	37%
45A6-7;45E2-3	4966	83	140	223	37%
56F11;56F16	7896	56	94	150	37%
42B3-5;43E15-18	1888	56	91	147	38%
76B4;77B	5126	42	67	109	39%
74D3-75A1;75B2-5	6411	53	84	137	39%
68A2-3;69A1-3	2611	38	60	98	39%
48C5-D1;48D5-E1	7145	67	102	169	40%
48E;49A	4960	56	85	141	40%
82F3-4;82F10-11	4787	59	87	146	40%
83C1-2;84B1-2	1990	41	59	100	41%
85C4-9;85D12-14	6756	61	85	146	42%
56D7-E3;56F9-12	6647	67	92	159	42%
66F1-2;67B2-3	7079	192	262	454	42%
60B9;60C4	9691	76	103	179	42%
76A7-B1;76B4-5	198	61	81	142	43%
98B1-2;98B3-5	7412	129	169	298	43%
44F10;45D9-E1	3591	48	60	108	44%
66D10-11;66E1-2	3024	51	63	114	45%
67A2;67D11-13	997	47	58	105	45%
95A4;95B1	7674	45	55	100	45%
59B;59D8-E1	7273	59	72	131	45%
50D1;50D2-7	6516	101	120	221	46%
45D3-4;45F2-6	6917	64	75	139	46%
50D4;50E4	7875	105	120	225	47%
95A5-7;95D6-11	2585	99	111	210	47%
48E12-F4;49A11-B6	5879	79	88	167	47%
54C8-D1;54E2-7	7441	98	109	207	47%
42E;44C	3368	59	64	123	48%
69D4-5;69F5-7	6456	62	67	129	48%
66E1-6;66F1-6	4500	43	45	88	49%
71C2-3;72B1-C1	6551	67	67	134	50%
79E5-F1;80A2-3	6649	32	31	63	51%
95A5-7;95C10-11	4940	58	52	110	53%
46C;47A1	1702	84	72	156	54%
50E6-F1;51E2-4	6455	47	36	83	57%

- Screened ~75% of the *Drosophila* genome (156 *Df* stocks)
- 36 Suppressors, 30 enhancers
- Secondary screening for individual gene suppressors/enhancers
- Microarry analysis of wild-type versus PAX-FKHR muscle: ~750 genes differentially expressed
  - Misexpressed genes = gene targets?
  - Unaltered genes potential co-factors

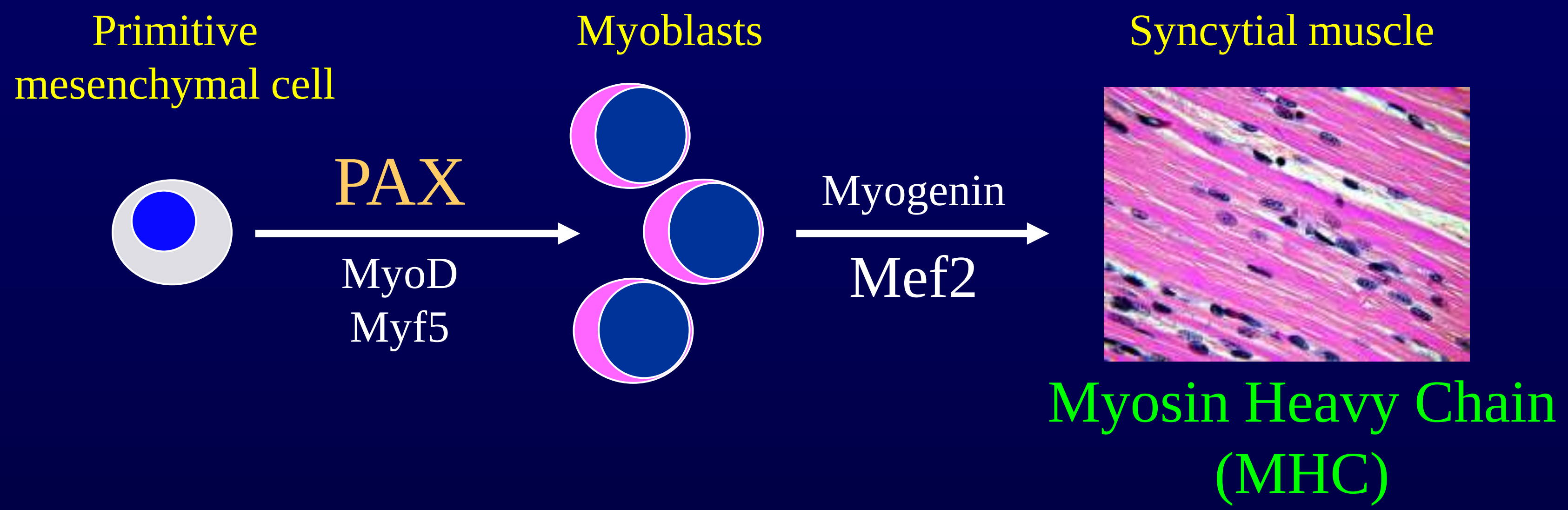
Molecular Effectors of PAX-FKHR Pathogenicity

Molecular Effectors of PAX-FKHR Pathogenicity



1. Muscle Development effectors/regulators
   - ✓ *Mef2*
2. Myoblast fusion regulators
   - ✓ *Antisocial*
3. Growth factor signaling
   - ✓ *EGFR*

Myogenesis: from precursor cell to syncytial muscle

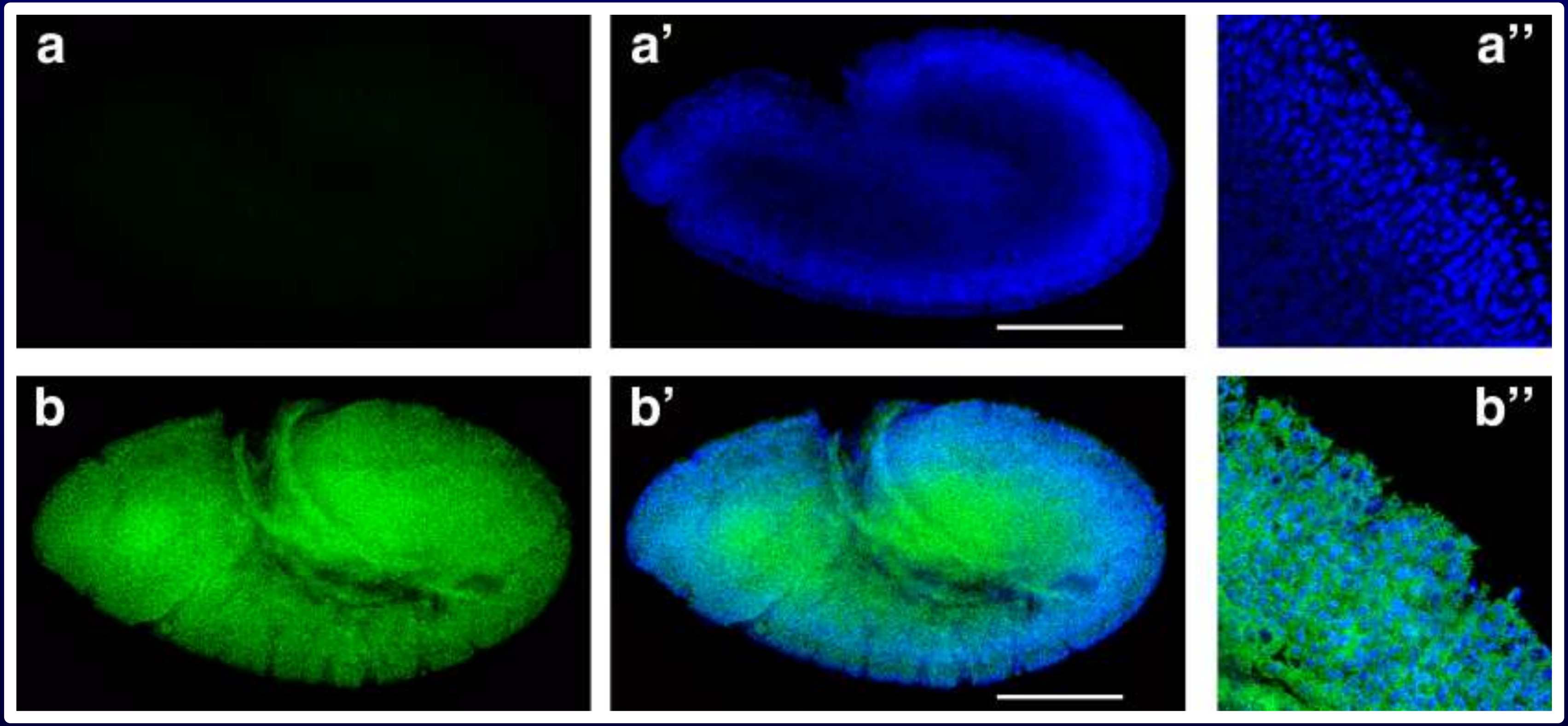


- Hypothesis- Human PAX-FKHR drives myogenesis in fly tissues
- Experiment:
  - Ubiquitous expression (*daughterless-Gal4>>UAS-PAX-FKHR*) of PAX-FKHR during embryogenesis
  - Test for MHC expression (MHC-GFP reporter)

Human PAX-FKHR Drives Myogenesis in *Drosophila* Embryos

wild-type, MHC-GFP

PAX-FKHR, MHC-GFP

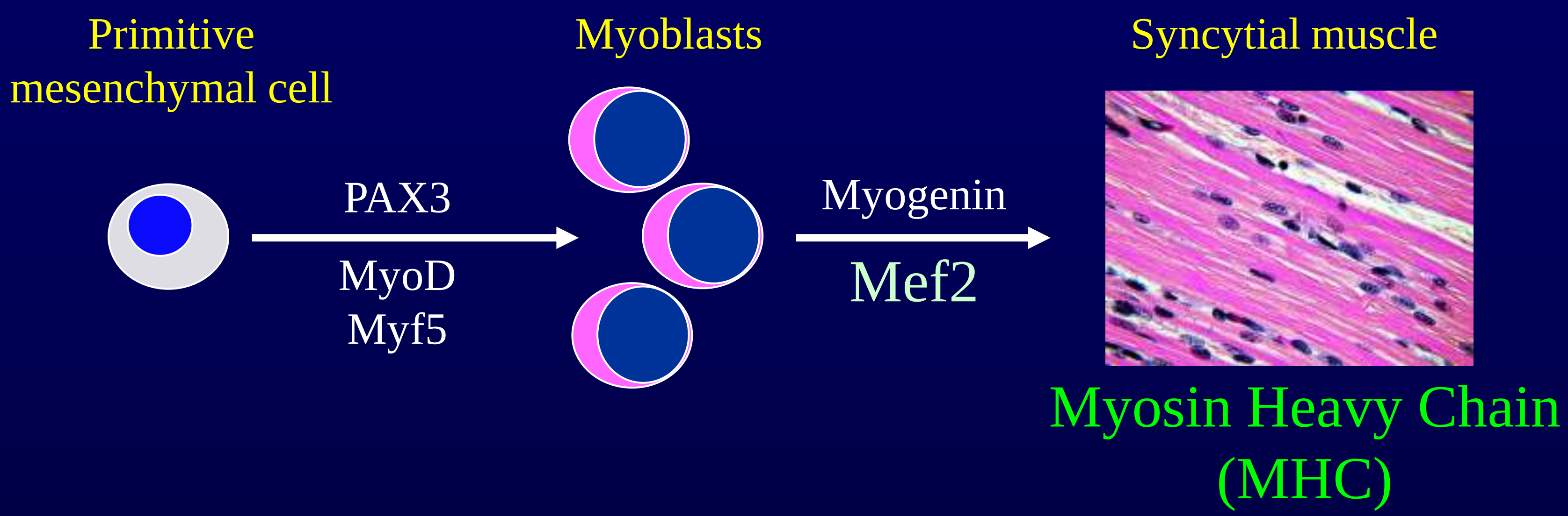


PAX-FKHR=daughterless-Gal4; UAS-PAX-FKHR

Blue=DAPI

Green=anti-GFP immunofluorescence

Myogenesis: from precursor cell to syncytial muscle



- *Mef2* is a critical gene in the myogenesis transcription factor cascade, and the master regulator of myogenesis in flies
- Hypotheses:
  - PAX-FKHR drives expression of *D-Mef2*
  - *D-Mef2* mutation will suppress PAX-FKHR pathogenicity

PAX-FKHR Screening and *D-Mef2*

95F7;96A17-18	2363	0	194	194	0%
95D7-D11;95F15	4432	0	225	225	0%
61A;61D3	2577	0	115	115	0%
75A6-7;75C1-2	2608	0	217	217	0%
64C;65C	3096	0	53	53	0%
75F10-11;76A1-5	6754	0	77	77	0%
56F9-17;57D11-12	3467	1	75	76	1%
85A2;85C1-2	1962	3	189	192	2%
70C1-2;70D4-5	3124	1	50	51	2%
49C1-4;50C23-D2	442	2	88	90	2%
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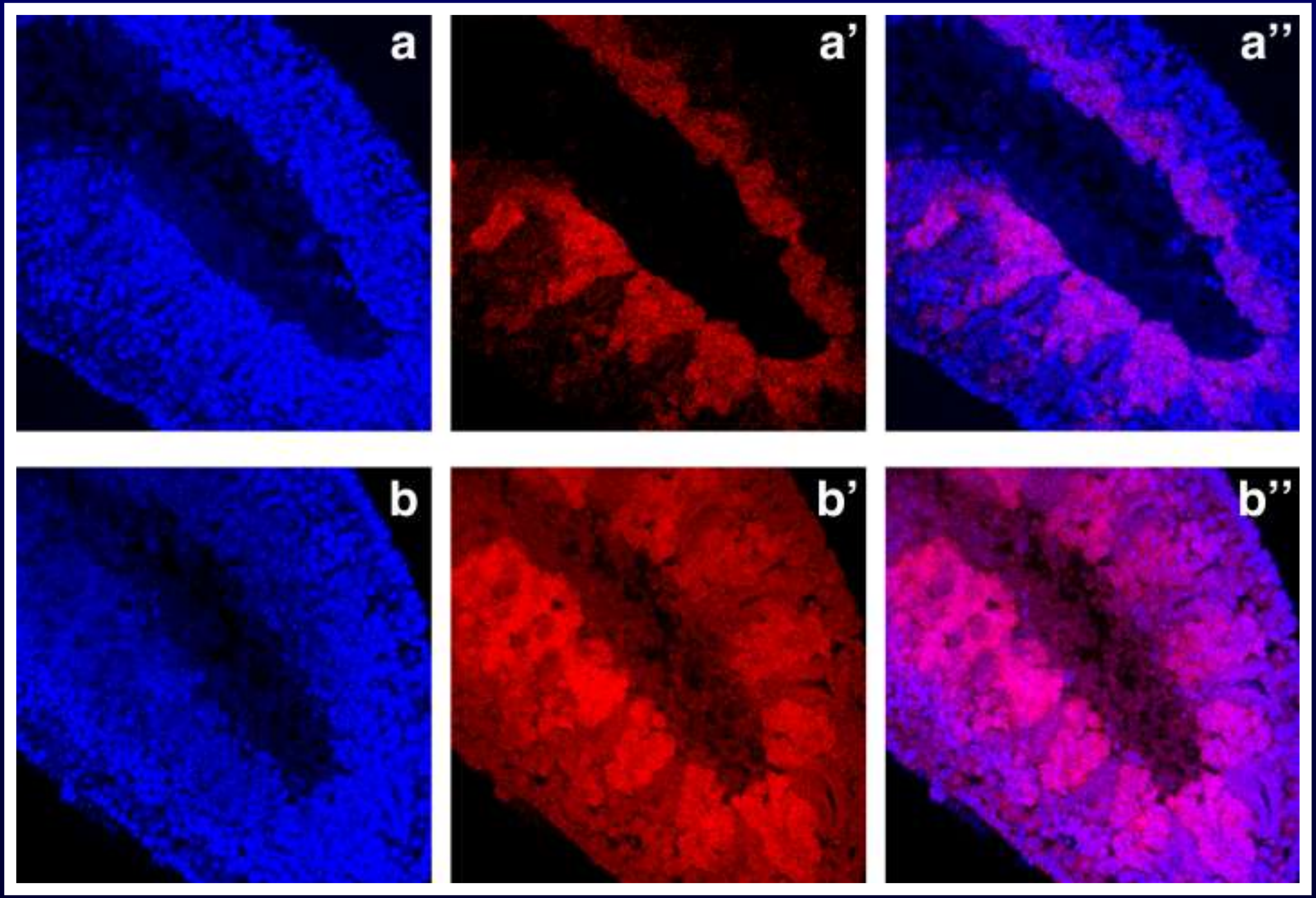
63F6-7;64C13-15	3686	4	97	101	4%
94A3-4;94D1-4	2586	3	71	74	4%
49A4-13;49E7-F1	754	6	128	134	4%
93B6-7;93D2	3340	5	98	103	5%
57D2-8;58D1	5246	8	140	148	5%
88E7-13;89A1	383	13	175	188	7%
54B2;54B17	9596	5	66	71	7%
81F3-6;82F5-7	1518	9	108	117	8%
60C8;60E8	9069	30	332	362	8%
53D9-E1;54B5-10	7445	12	112	124	10%
54C1-4;54C1-4	5574	20	180	200	10%
85D8-12;85E7-F1	1931	19	170	189	10%
97E3;98A5	823	18	152	170	11%
41A;41A	739	22	167	189	12%
60F1;60F5	4961	11	80	91	12%
66B8-9;66C9-10	1541	19	129	148	13%
63E6-9;64A8-9	463	42	276	318	13%
84A5;84D9	1842	18	106	124	15%
82F8-10;83A1-3	5694	34	196	230	15%
62A10-B1;62D2-5	600	14	80	94	15%
96F1;96F10	9500	17	93	110	15%
77B-C;77F-78A	3127	35	190	225	16%
59D5-10;60B3-8	1682	18	94	112	16%
44D1-4;44F12	201	38	196	234	16%
56F12-14;57A4	6609	14	71	85	16%
99B5-6;99F1	3547	51	254	305	17%
95D8;95E5	7676	22	109	131	17%
99D3;3Rt	3546	41	197	238	17%
???	447	17	81	98	17%
50E4;50F6	7876	29	136	165	18%
96C9;96E2	7681	32	150	182	18%
77F3;78C8-9	4429	83	387	470	18%
67E3-7;68A2-6	6471	21	96	117	18%
48A3-4;48C6-8	1145	22	97	119	18%
96F1;97B1	5601	28	123	151	19%
84A1-2;84B1-2	1884	26	114	140	19%
83B7-C1;83C6-D1	7443	32	140	172	19%
51D3-8;52F5-9	3518	27	118	145	19%
78D5;79A2	8101	93	390	483	19%
42A1-2;42E6-F1	1007	28	116	144	19%
83E1-2;84A5	2393	19	78	97	20%
	w1118	98	390	488	20%
71F1-4;72D1-10	3640	72	278	350	21%
97F1-2;98A	9529	46	176	222	21%
62A2;62A7	7566	103	389	492	21%
54B17-C4;54C1-4	5680	34	126	160	21%
77E2-4;78A2-4	5878	44	163	207	21%
	w1118	75	272	347	22%
73A3;74F	2998	39	134	173	23%
96E2;96E6	7682	37	160	164	23%
98E3;99A6-8	430	63	210	273	23%
95A2-4;95A8-B1	9497	43	141	184	23%
94E9;94E13	7990	37	118	155	24%
95C12;95D8	7675	52	155	207	25%
	w1118	74	218	292	25%
54B1-2;54B7-10	7414	47	133	180	26%
60C5-6;60D9-10	2604	39	109	148	26%
66A17-20;66C1-5	5877	23	62	85	27%
68C8-11;69B4-5	2612	47	126	173	27%
86C1;87B1-5	3128	38	99	137	28%
77A1;77D1	2052	58	150	208	28%
77A1;77D1	8082	35	90	125	28%
75F2;76A1	757	31	79	110	28%
69A4-5;69D4-6	5492	43	107	150	29%
58D1-2;59A	282	40	92	132	30%
83B4;83B6	8103	50	104	154	32%
55A;55F	1547	71	147	218	33%
95B1;95D1	7992	53	109	162	33%
69F6-70A1;70A1-2	6457	36	74	110	33%
98E3;99A6-8	430	38	78	116	33%
45F6;46B12	9410	43	86	129	33%
59A1-3;59D1-4	3909	43	85	128	34%
97A;98A1-2	1910	90	176	266	34%
65F3;66B10	1420	41	79	120	34%
56C4;56D6-10	6866	48	88	136	35%
72C1-D1;73A3-4	2993	58	106	164	35%
98E3;99A6-8	430	39	70	109	36%
62B4-7;62D5-E5	2400	51	89	140	36%
46A;46C	1743	50	86	136	37%
54D1-2;54E5-7	6779	58	99	157	37%
45A6-7;45E2-3	4966	83	140	223	37%
56F11;56F16	7896	56	94	150	37%
42B3-5;43E15-18	1888	56	91	147	38%
76B4;77B	5126	42	67	109	39%
74D3-75A1;75B2-5	6411	53	84	137	39%
68A2-3;69A1-3	2611	38	60	98	39%
48C5-D1;48D5-E1	7145	67	102	169	40%
48E;49A	4960	56	85	141	40%
82F3-4;82F10-11	4787	59	87	146	40%
83C1-2;84B1-2	1990	41	59	100	41%
85C4-9;85D12-14	6756	61	85	146	42%
56D7-E3;56F9-12	6647	67	92	159	42%
66F1-2;67B2-3	7079	192	262	454	42%
60B9;60C4	9691	76	103	179	42%
76A7-B1;76B4-5	198	61	81	142	43%
98B1-2;98B3-5	7412	129	169	298	43%
44F10;45D9-E1	3591	48	60	108	44%
66D10-11;66E1-2	3024	51	63	114	45%
67A2;67D11-13	997	47	58	105	45%
95A4;95B1	7674	45	55	100	45%
59B;59D8-E1	7273	59	72	131	45%
50D1;50D2-7	6516	101	120	221	46%
45D3-4;45F2-6	6917	64	75	139	46%
50D4;50E4	7875	105	120	225	47%
95A5-7;95D6-11	2585	99	111	210	47%
48E12-F4;49A11-B6	5879	79	88	167	47%
54C8-D1;54E2-7	7441	98	109	207	47%
42E;44C	3368	59	64	123	48%
69D4-5;69F5-7	6456	62	67	129	48%
66E1-6;66F1-6	4500	43	45	88	49%
71C2-3;72B1-C1	6551	67	67	134	50%
79E5-F1;80A2-3	6649	32	31	63	51%
95A5-7;95C10-11	4940	58	52	110	53%
46C;47A1	1702	84	72	156	54%
50E6-F1;51E2-4	6455	47	36	83	57%

- *Df(2R)X1* is a suppressor
- *Dmef2$^{22\text{-}21}$ (null allele)* strongly suppresses PAX-FKHR
- Microarry analysis of *wild-type* versus *PAX-FKHR* muscle: ~750 genes differentially expressed
  - Misexpressed genes = gene targets
  - Unaltered genes potential co-factors
- Other myogenesis regulators- e.g, *MyoD*, *muscleblind*

D-Mef2 is a PAX-FKHR Target Gene in *Drosophila* Embryos

wild-type, D-Mef2-GFP

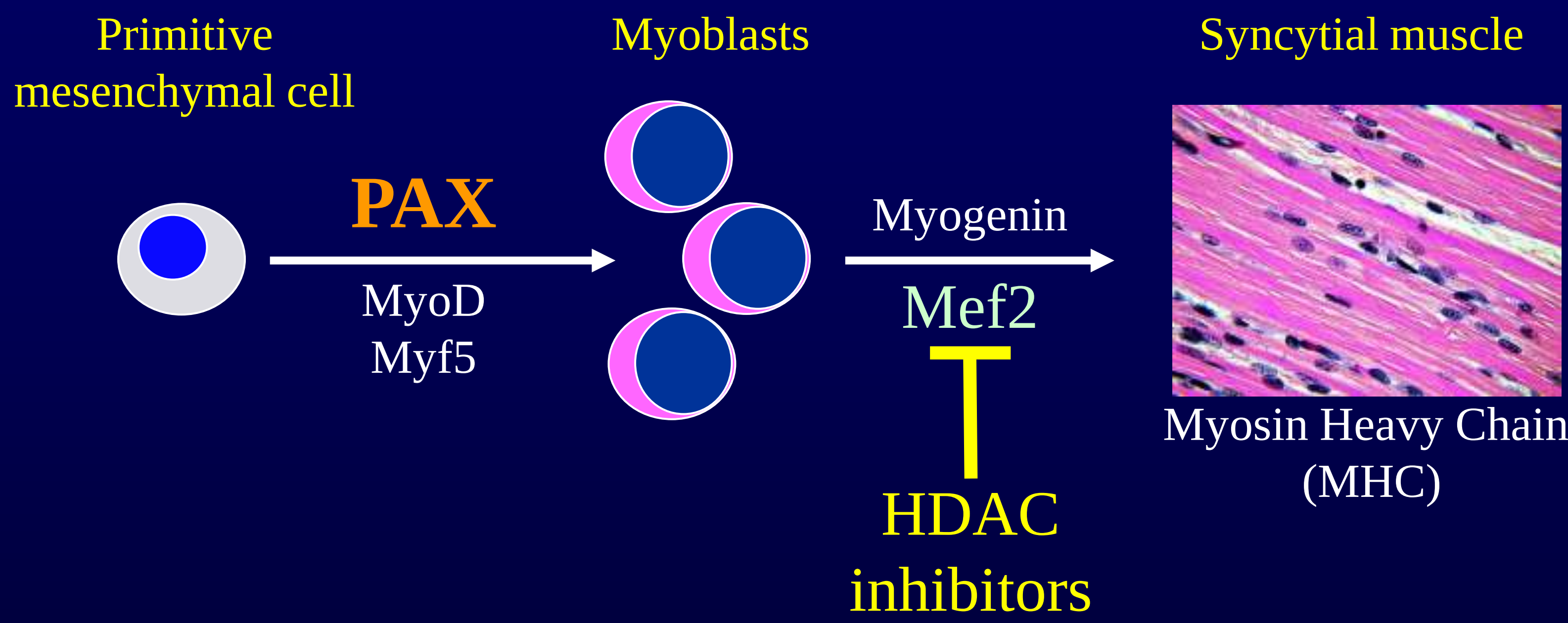
PAX-FKHR, D-Mef2-GFP



PAX-FKHR=daughterless-Gal4; UAS-PAX-FKHR

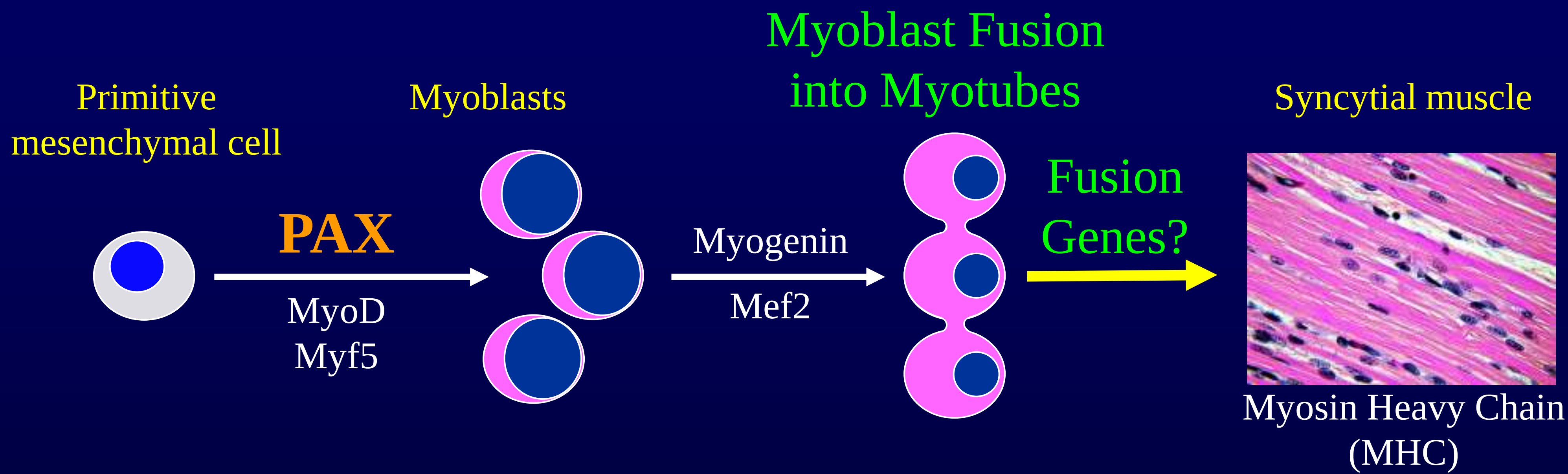
Blue=DAPI, Red=anti-GFP immunofluorescence

Myogenesis: from precursor cell to syncytial muscle



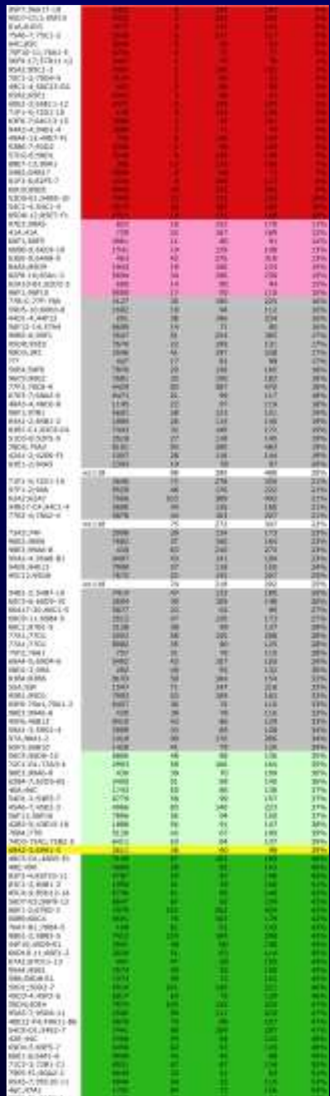
- *Mef2* involvement in RMS has not been reported
- "Gotta love the fly"- ONE Mef2 gene versus FOUR in mammals!
- Drugs that "poison" Mef2 activity (HDAC inhibitors) are new potential RMS therapies

Myogenesis: from precursor cell to syncytial muscle



- Myoblast fusion genes are required to form syncytial tissue
- Little is known regarding mammalian myoblast fusion genes
- Could fusion gene misregulation underlie RMS pathogenesis?

PAX-FKHR Screening and Results

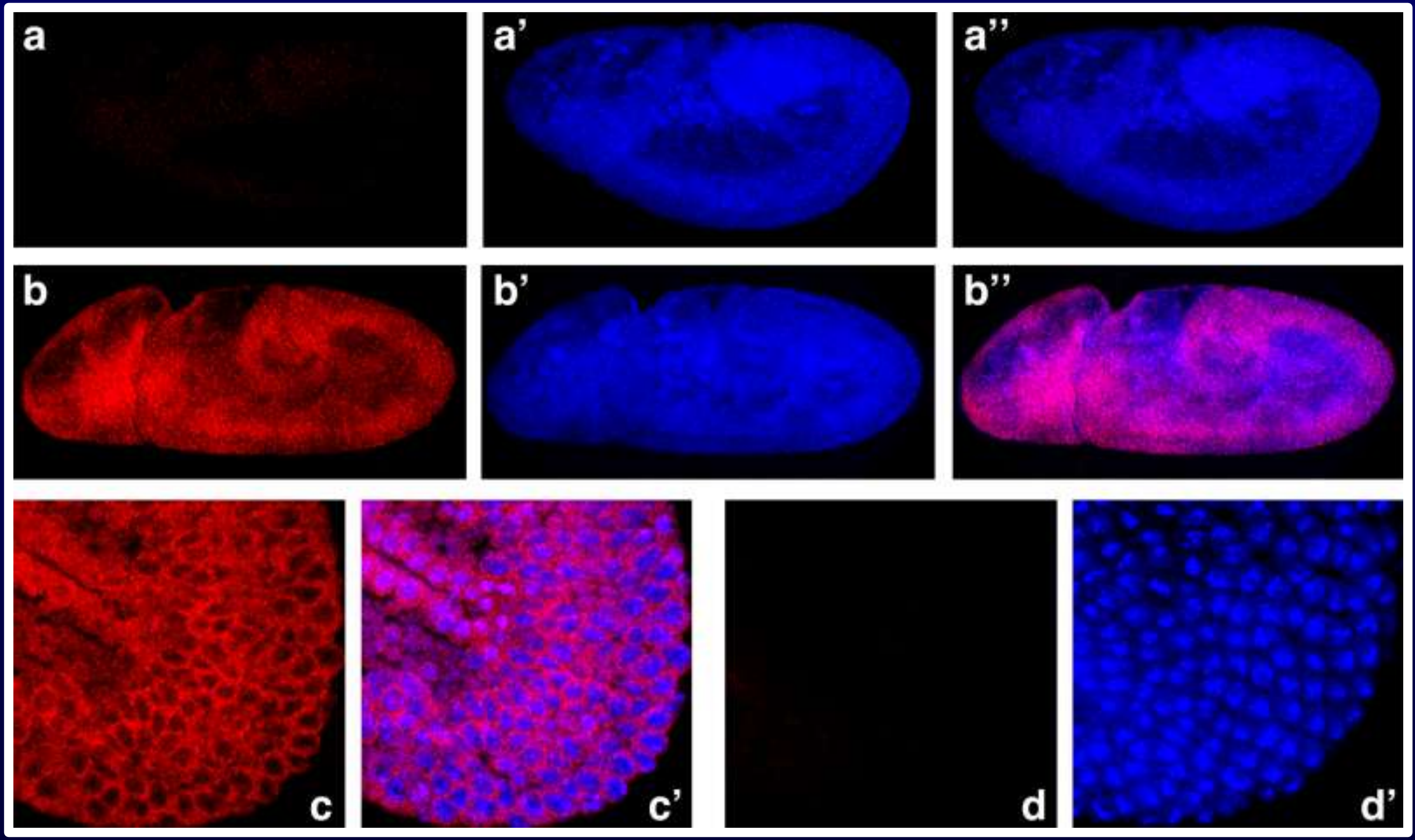


- *Df(3L)vin5* is a strong suppressor
- *antisocial (ants)*^{P1729} *(rols) LOF allele* strongly suppresses PAX-FKHR
- Microarry analysis of wild-type versus PAX-FKHR muscle: ~750 genes differentially expressed
    - Misexpressed genes = gene targets
    - Unaltered genes potential co-factors
- Other myoblast fusion regulators- e.g., *blown-fuse, myoblast city*

ants is a PAX-FKHR Target Gene

wild-type

PAX-FKHR

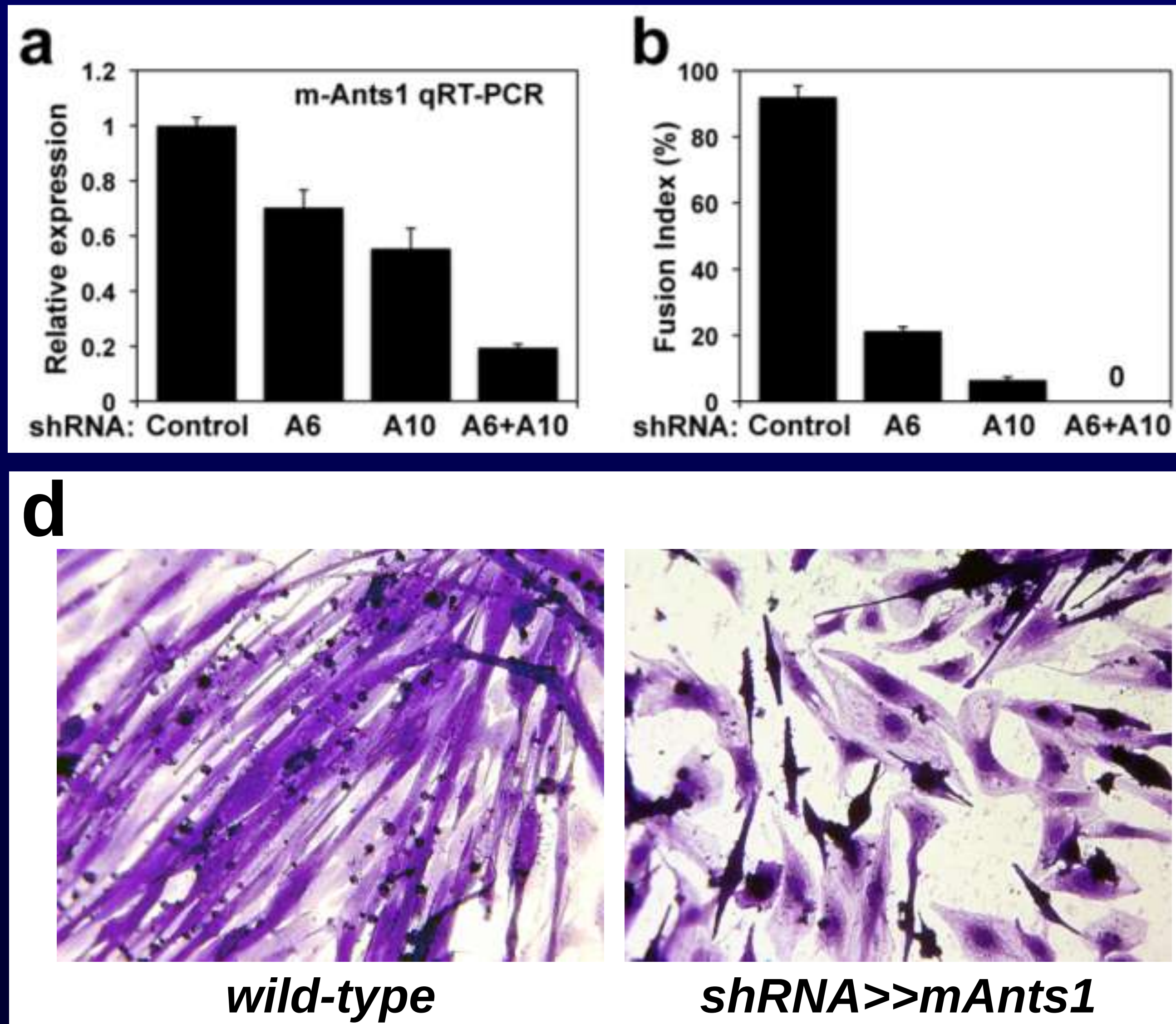


PAX-FKHR *wild-type*

PAX-FKHR=daughterless-Gal4; UAS-PAX-FKHR | Blue=DAPI | Red=anti-Ants immunofluorescence

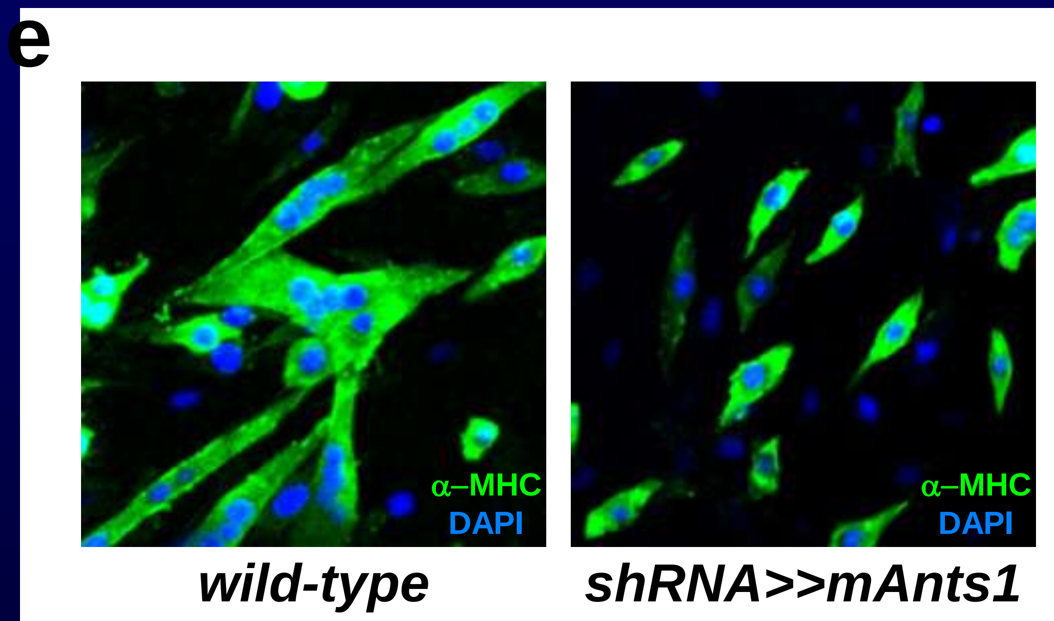
Avirneni-Vadlamudil *et al*. (2011), *Submitted*

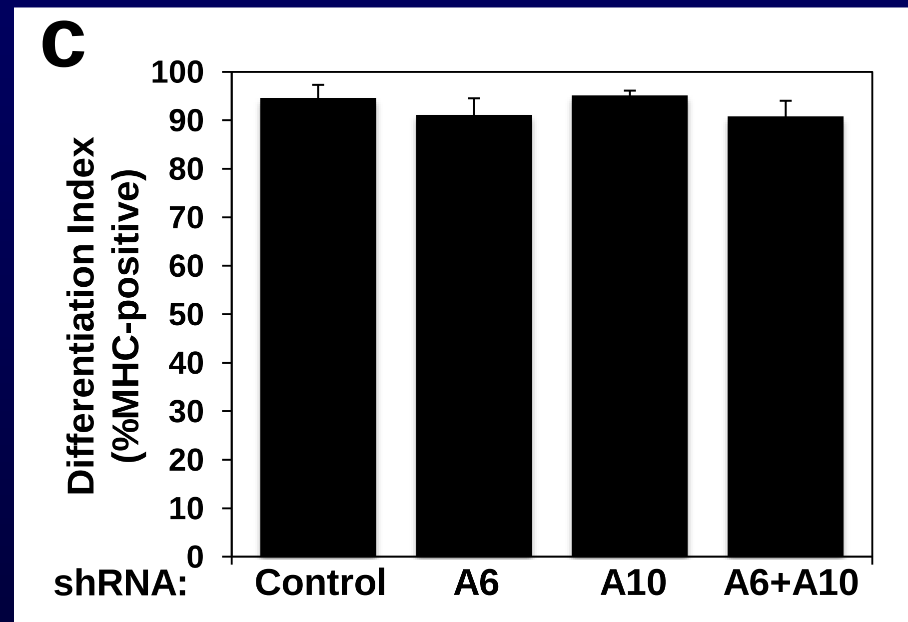
Ants1 (Tanc1) is a Mammalian Myoblast Fusion Gene



Avirneni-Vadlamudil *et al*. (2011),

Ant1 is Not Required for Differentiation

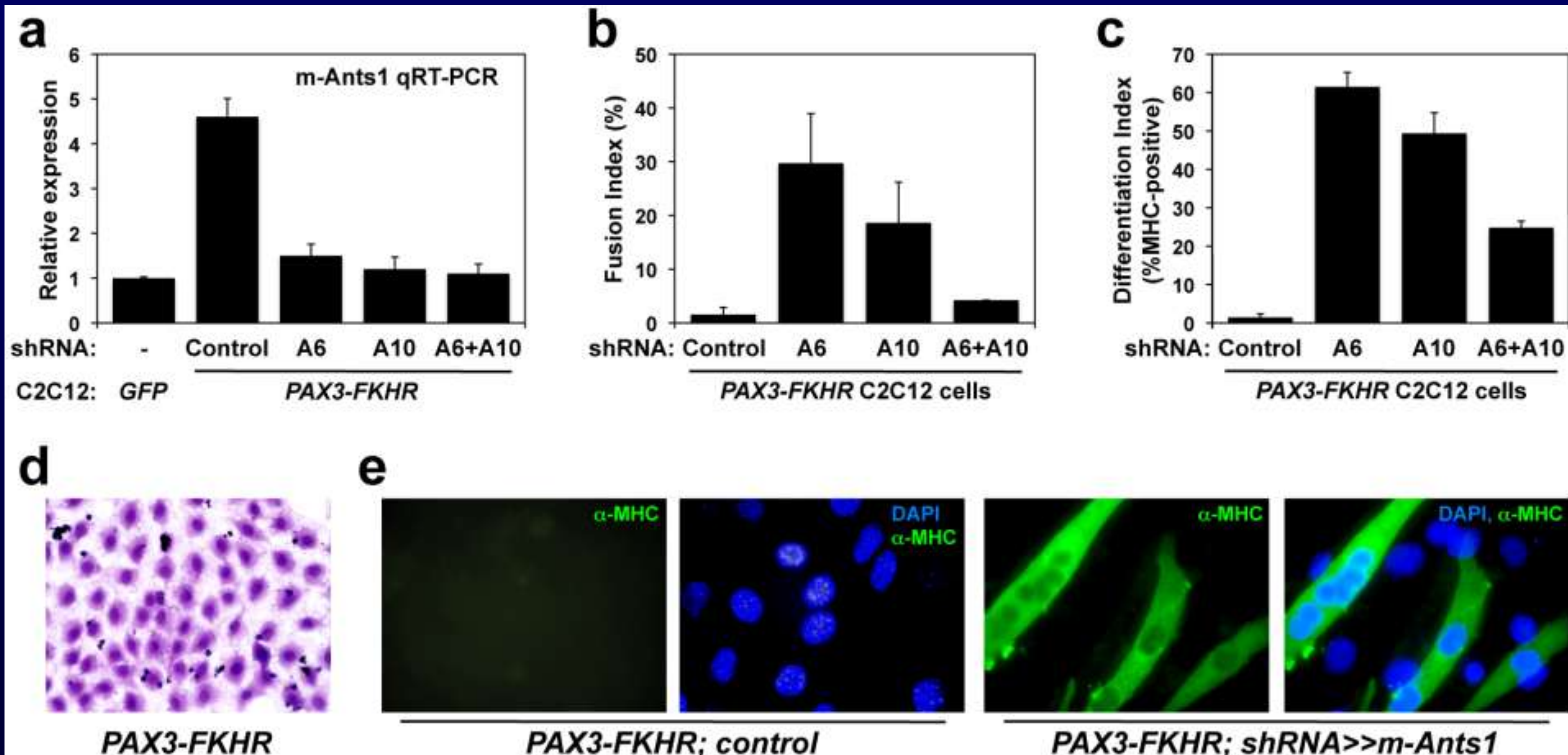




anti-Myosin Heavy Chain (MHC)

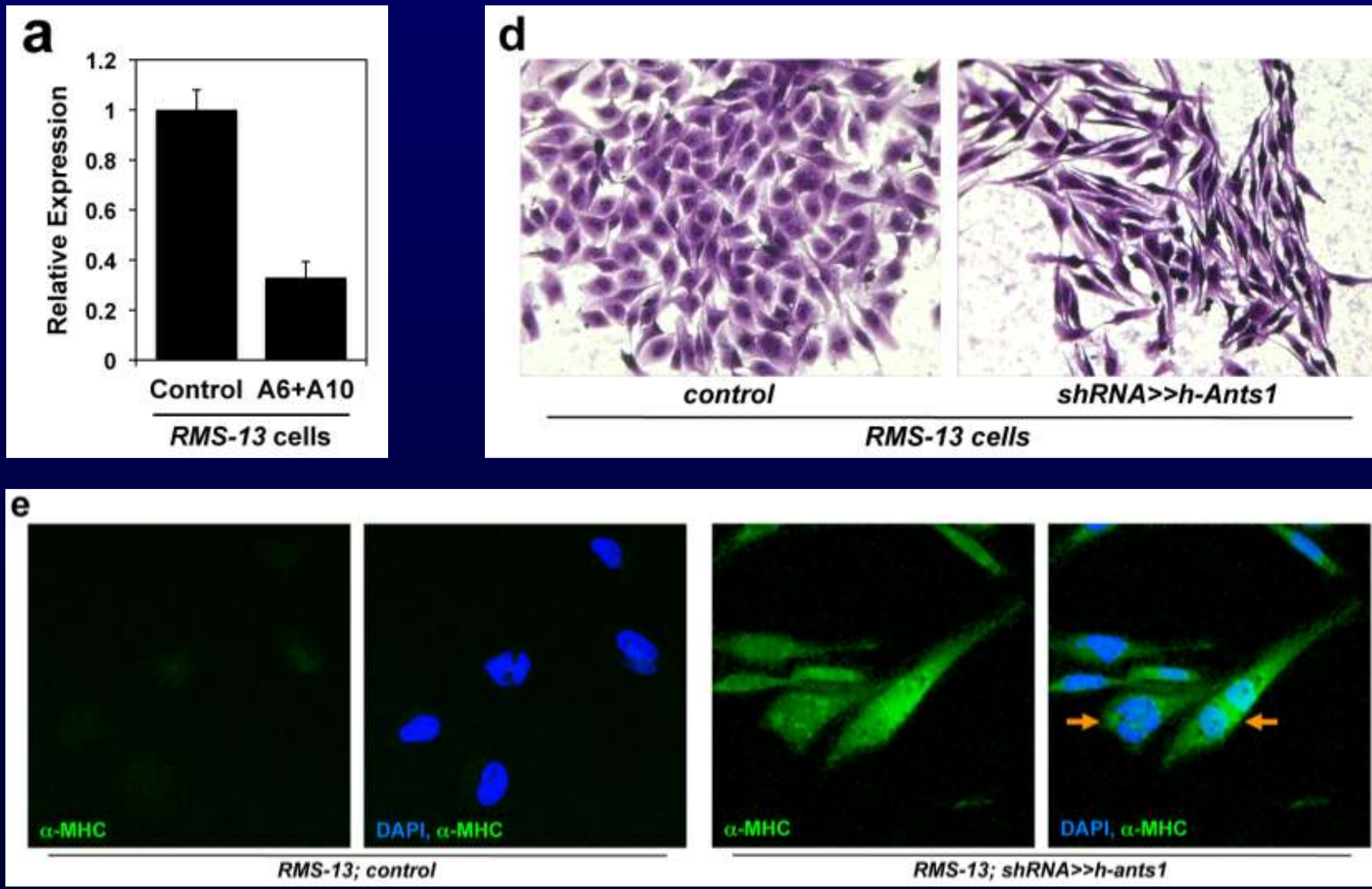
Avirneni-Vadlamudil *et al*. (2011),

Ants1-Silencing Suppresses PAX-FKHR Pathogenicity in Mammalian Myoblasts

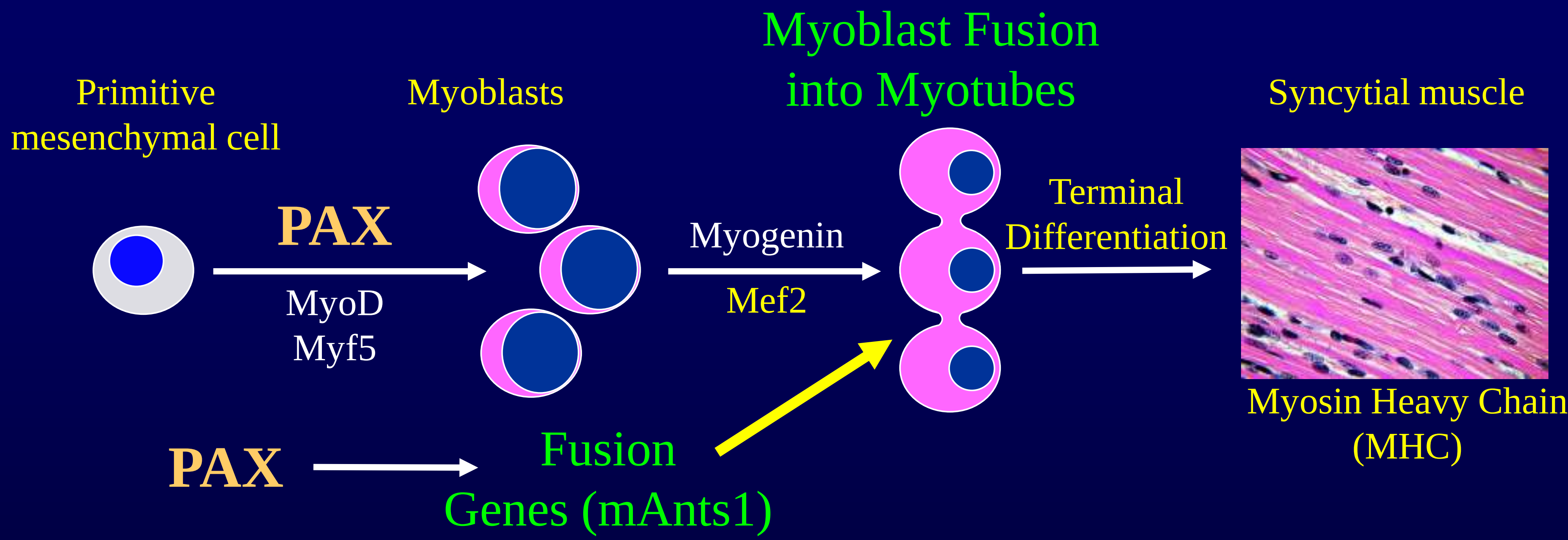


Avirneni-Vadlamudil *et al*. (2011),

Ants1-Silencing Rescues PAX-FKHR RMS Cells from Differentiation-Arrest and Fusion Failure

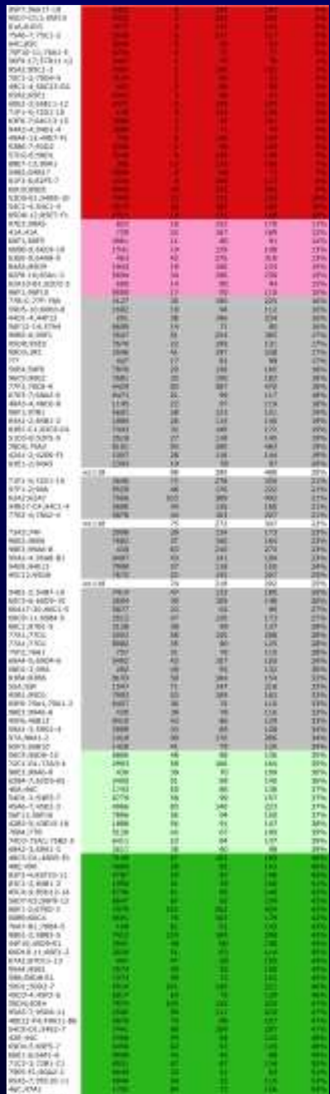


Myogenesis, RMS Pathogenesis, and *Ants1*



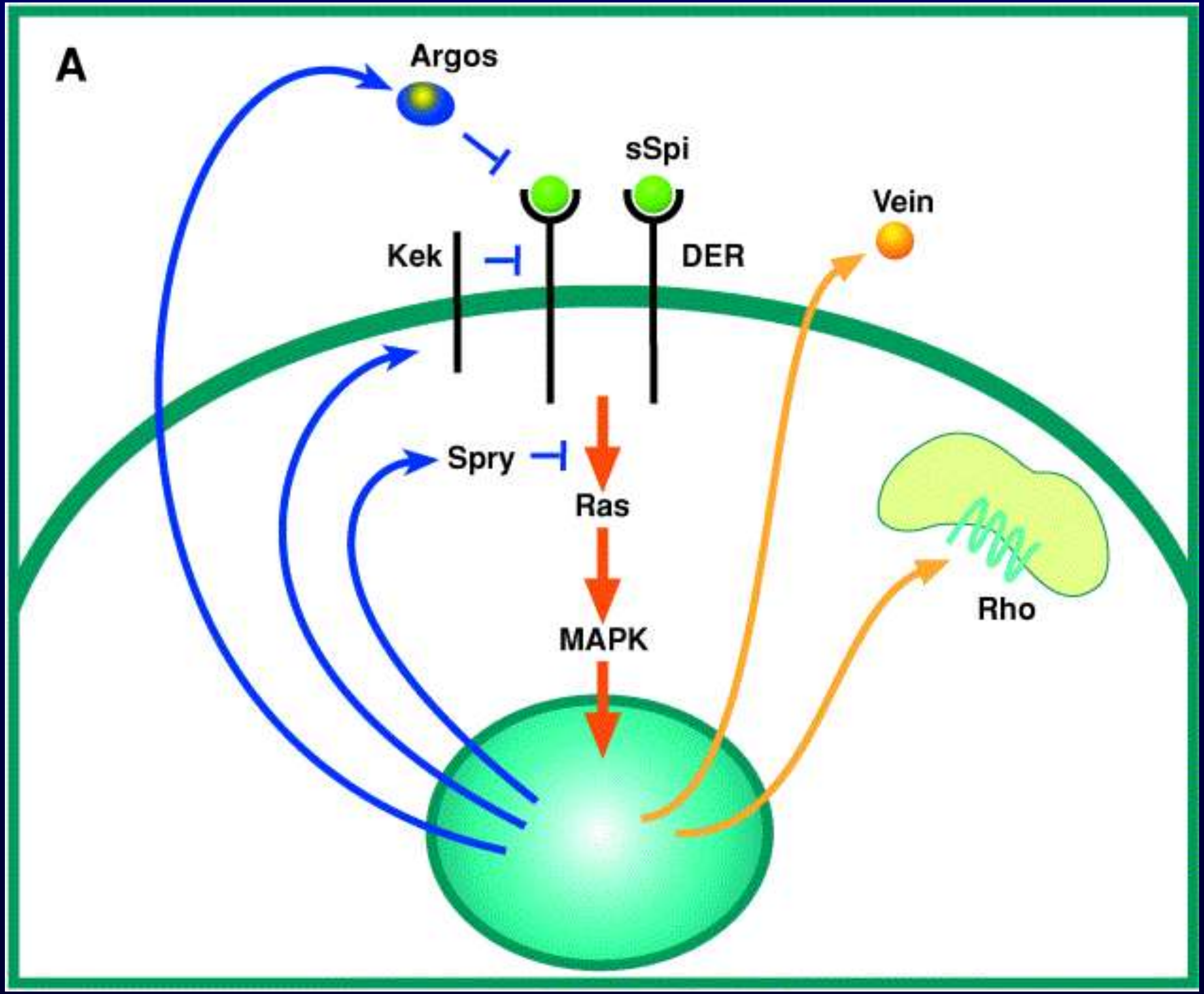
- *Ants1* is a PAX-FKHR target and mammalian myoblast fusion gene
- Correction of *Ants1* expression rescues RMS terminal differentiation arrest; mAtns is a new putative RMS disease gene
- *In Vivo* profiling: xenografts, gene sequencing, CGH

PAX-FKHR Screening and Results



- Ras is a PAX-FKHR genetic interactor
- Microarry analysis of wild-type versus PAX-FKHR muscle: ~750 genes differentially expressed
  - Misexpressed genes = gene targets
  - Unaltered genes = potential co-factors
- Regulators of Epidermal Growth Factor signaling (*i.e.*, *EGFR* ligand, *EGFR* inhibitor, *EGFR*)

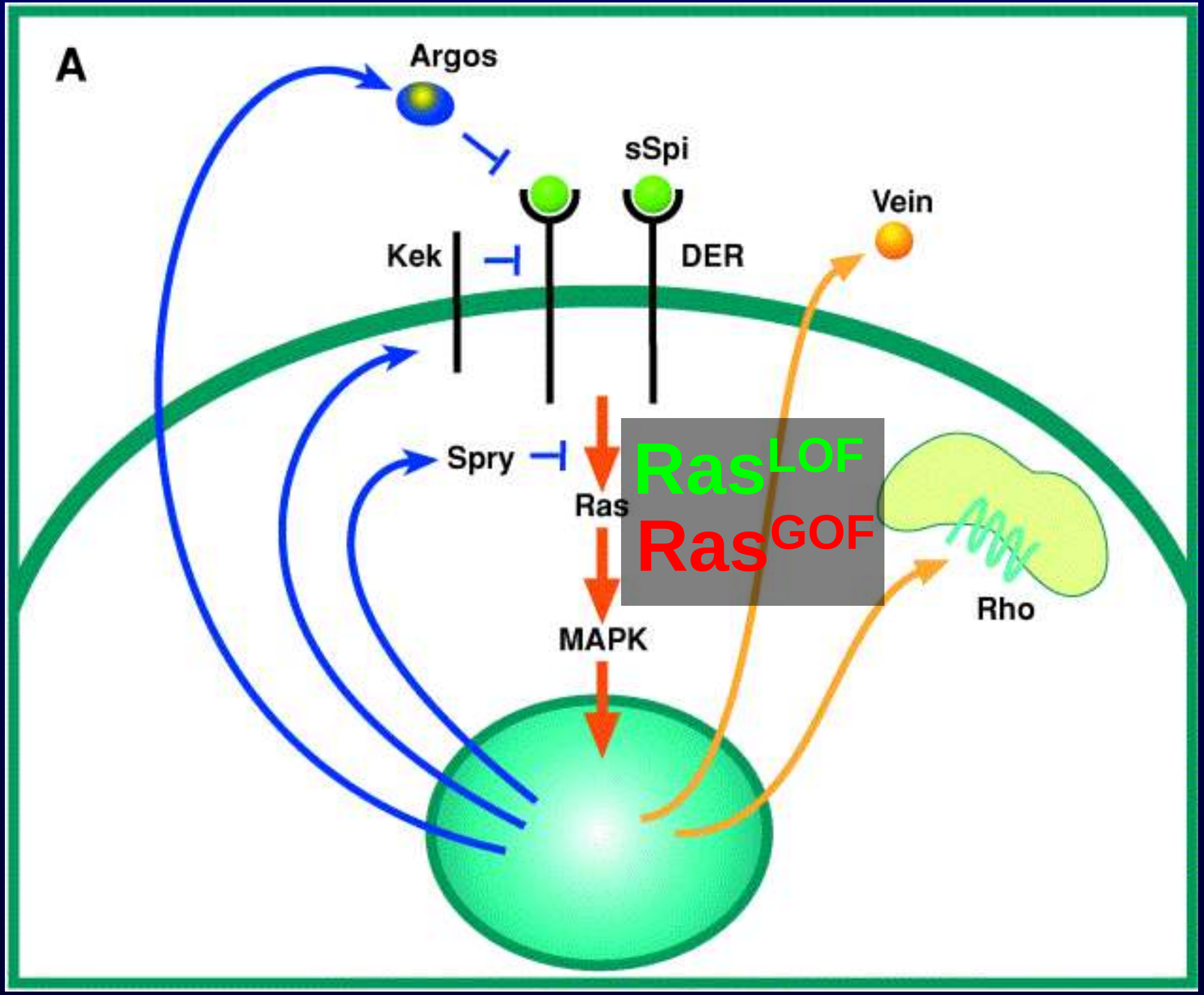
Epidermal Growth Factor Receptor Signaling and PAX-FKHR



- ✓ DER = EGF Receptor
- ✓ sSpi = EGFR Ligand
- ✓ Ras- intracellular effector
- ✓ Kek = Kekkon; EGFR inhibitor

http://www.weizmann.ac.il/molgen/members/shilo/new_pages/scienc_activities.html

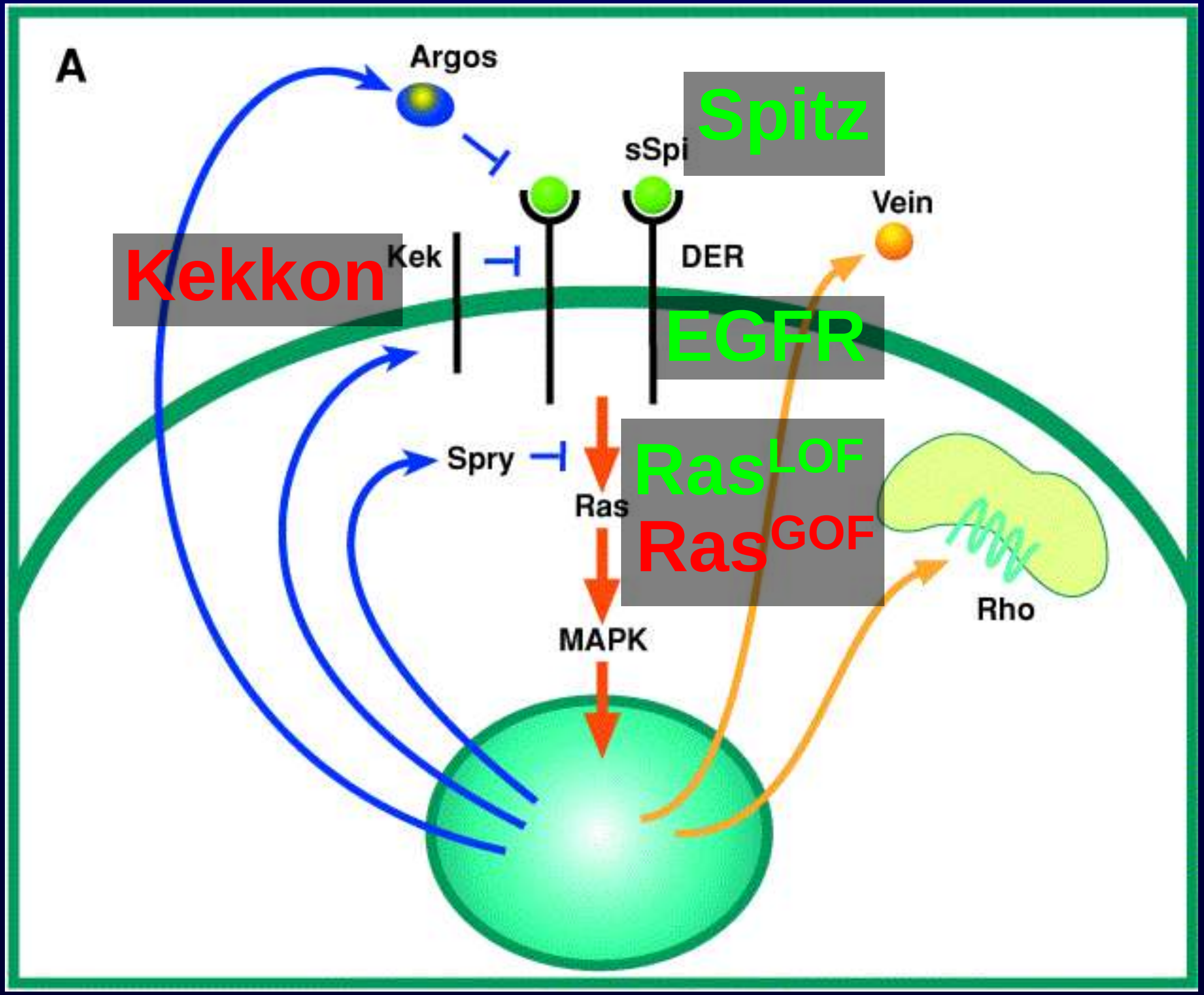
Epidermal Growth Factor Receptor Signaling and PAX-FKHR



- ✓ DER = EGF Receptor
- ✓ sSpi = EGFR Ligand
- ✓ Ras- intracellular effector
  - ✓ Ras^{LOF}
  - ✓ Ras^{GOF}
- ✓ Kek = Kekkon; EGFR inhibitor

http://www.weizmann.ac.il/molgen/members/shilo/new_pages/scienc_activities.html

Epidermal Growth Factor Receptor Signaling and PAX-FKHR



- ✓ DER = EGF Receptor
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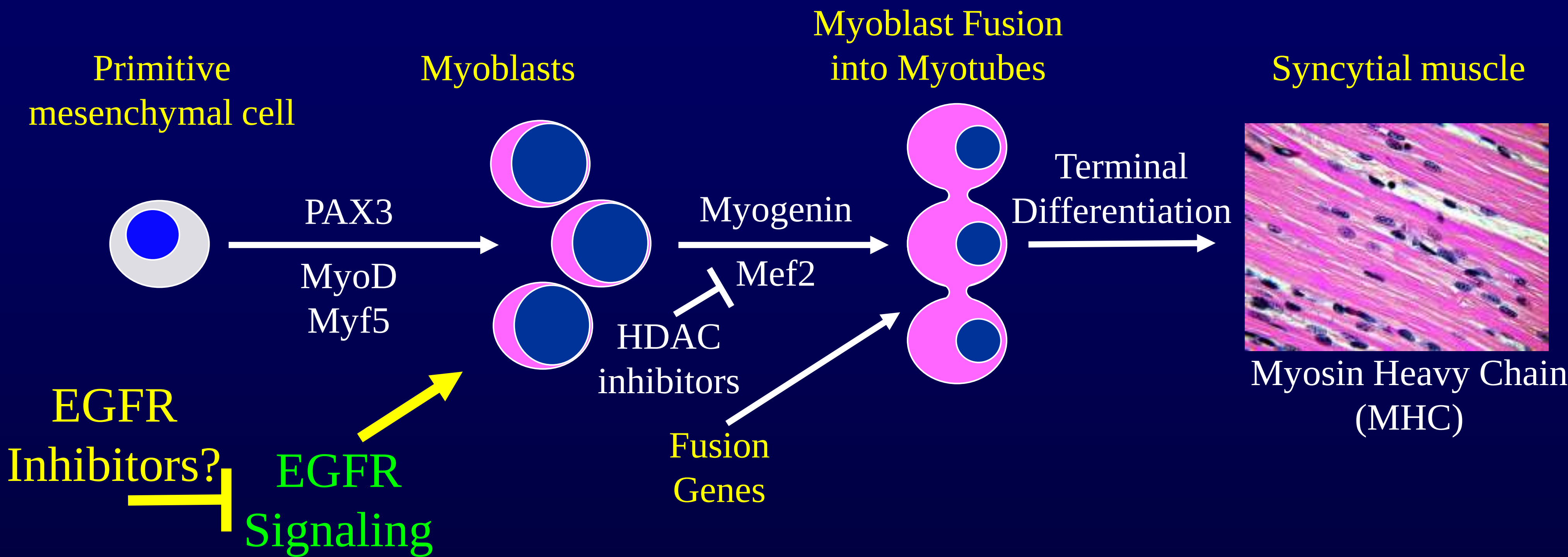
  - ✓ Ras^{LOF}
  - ✓ Ras^{GOF}
- ✓ Kek = Kekkon; EGFR inhibitor

http://www.weizmann.ac.il/molgen/members/shilo/new_pages/scienc_activities.html

Myogenesis: EGFR Signaling



- EGFR signaling participates in PAX-FKHR pathogenic activity
- Suggests that directed therapy targeting EGFR (Tarceva) might be a new, effective RMS therapy

Tarceva Therapy Blocks PAX-FKHR Pathogenicity *in vivo*

Summary and Future Studies

- Utilizing a *Drosophila* model system to identify new PAX-FKHR co-factors and gene targets
  - Genetic screening
  - microarray analysis

- Isolating:
  - Muscle development regulators: *Mef2*
  - Growth factor signaling: *EGFR*
  - Myoblast fusion elements: *Antisocial*

Summary and Future Studies

- Characterize PAX-FKHR co-factors/gene targets for activity in mammalian systems
  - Mammalian myoblasts
  - Tumor tissue/cell lines: *Gene Sequencing and Protein Expression*
  - Mouse RMS transgenic model

- Long term goals:
  - Drug screening
  - Tumor diagnosis and prognosis
  - General themes in pediatric sarcoma tumorigenesis?

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