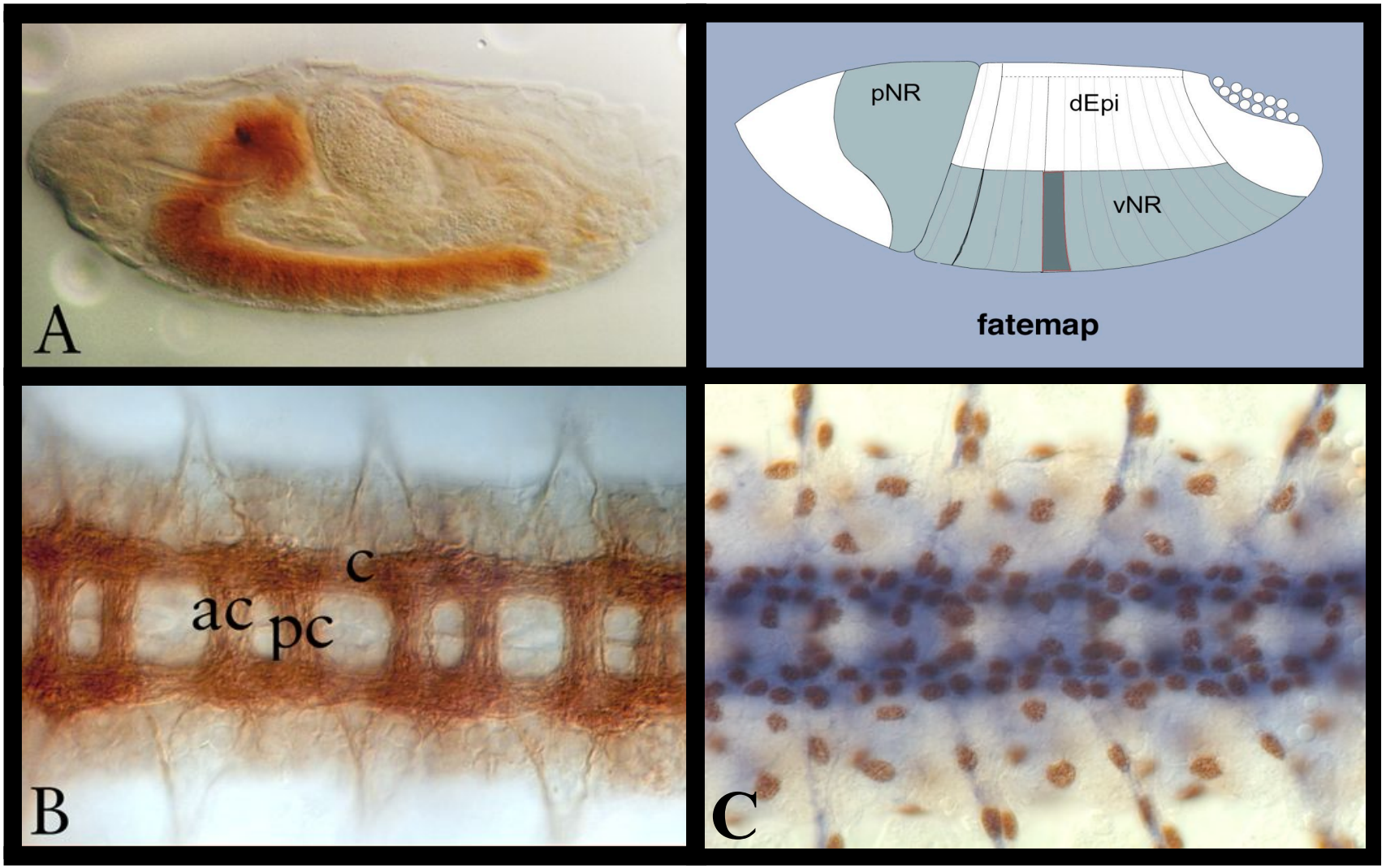
Gene expression profiling during gliogenesis in the *Drosophila* embryo

Angela Becker

Introduction to the modellorganism *Drosophila melanogaster*

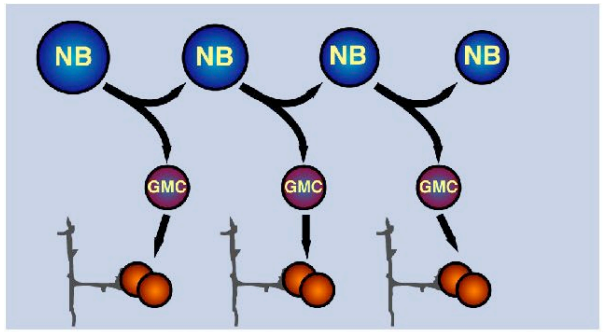
- **the embryonic development**
- **structure and development of the central nervous system (CNS)**
- **neural stem cells and the regulation of cell fate choice**

The embryonic CNS of *Drosophila*

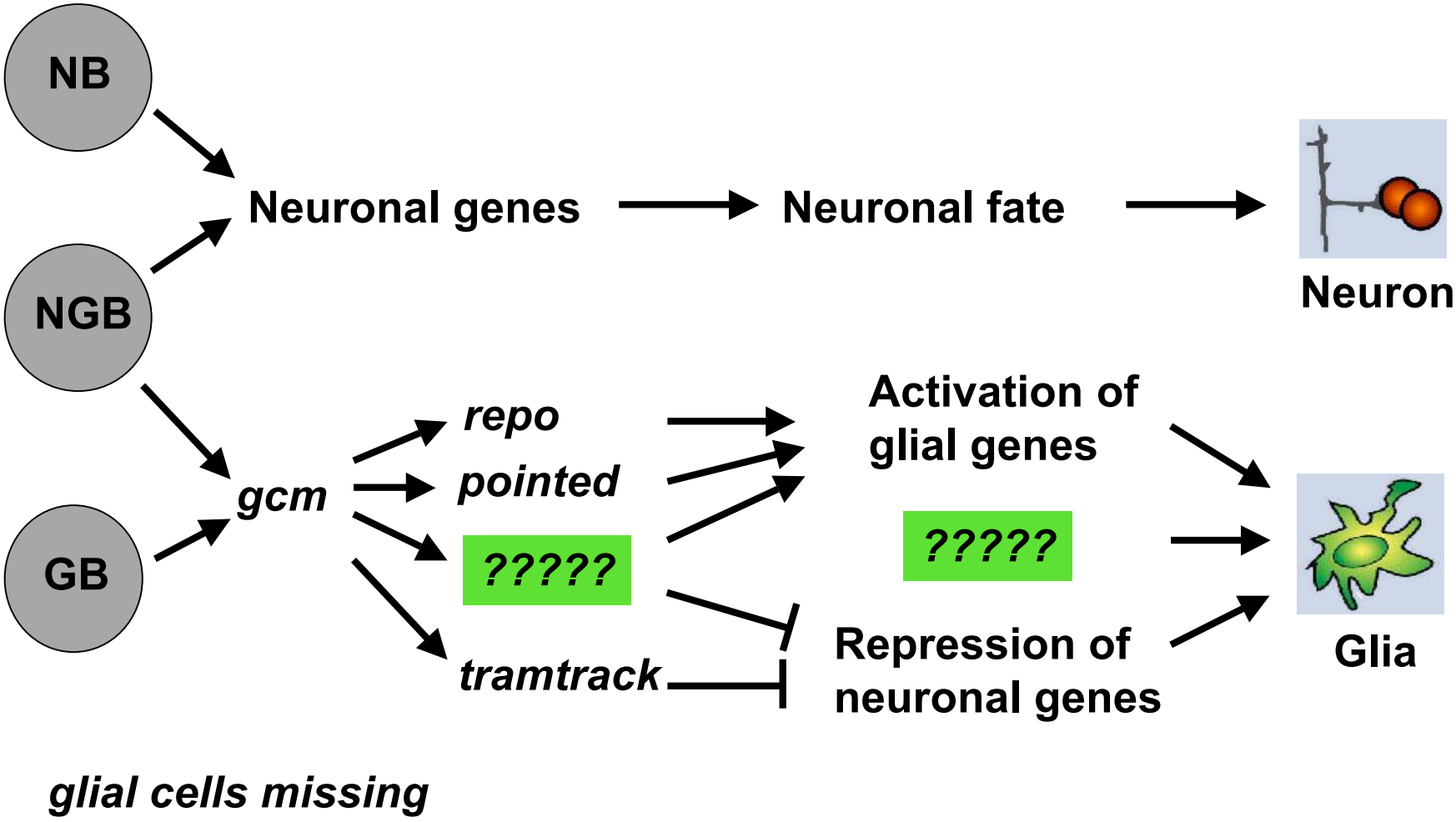


Three types of neural precursor cells in the CNS

Neuroblast



Regulation of cell fate choice



glial cells missing

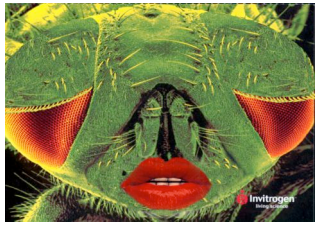
Design and performance of a dual microarray approach

Aim: Identification of *gcm*-dependent target genes

- **Genome-wide search based on microarray-experiments**

Microarray Experiments

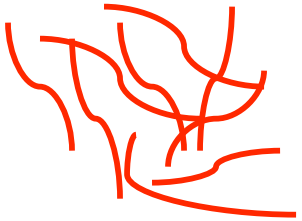
„wildtype situation“



„mutant situation“

RNA

Cy3



labelled cDNA

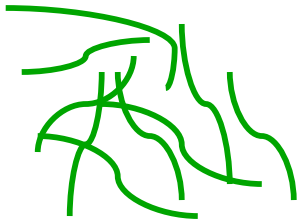
Preparation of total RNA (targets)

Reverse transcription of mRNA

Labelling with fluorescent dyes

RNA

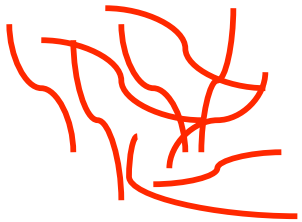
Cy5

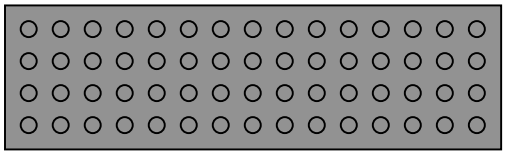


labelled cDNA

„wildtype situation“

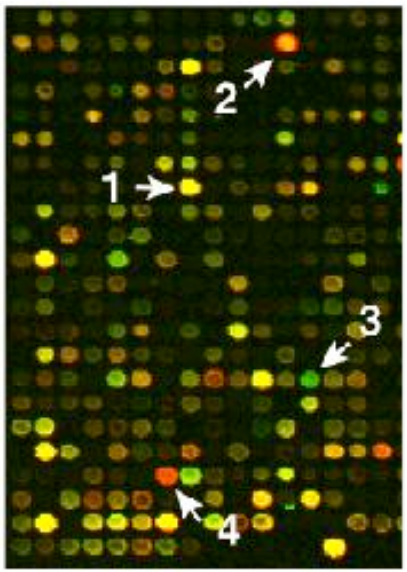
„mutant situation“





Hybridization on microarrays

Scanning the slide

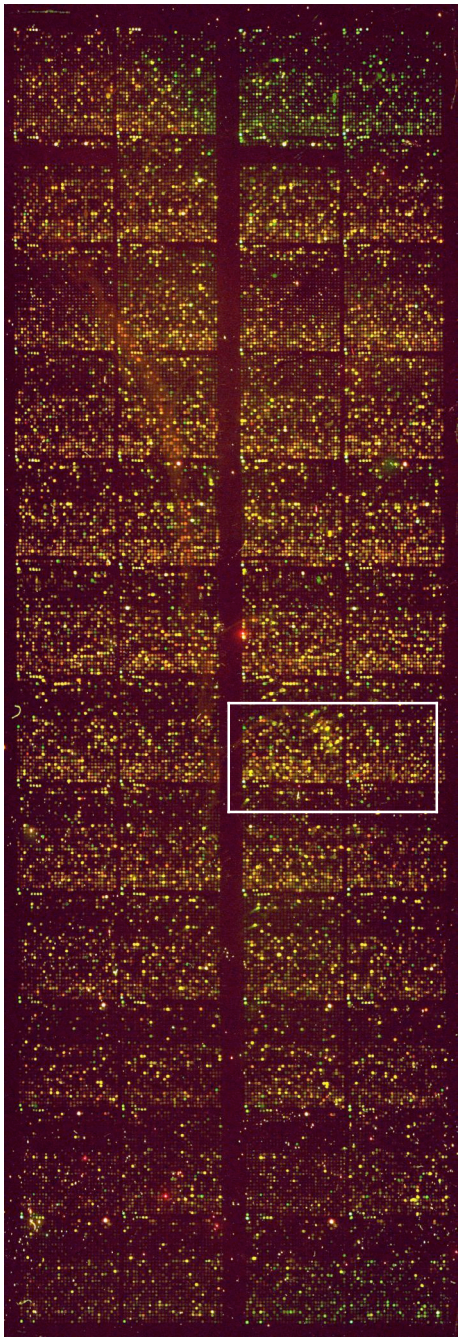


expressed in wildtype

expressed in both

expressed in both

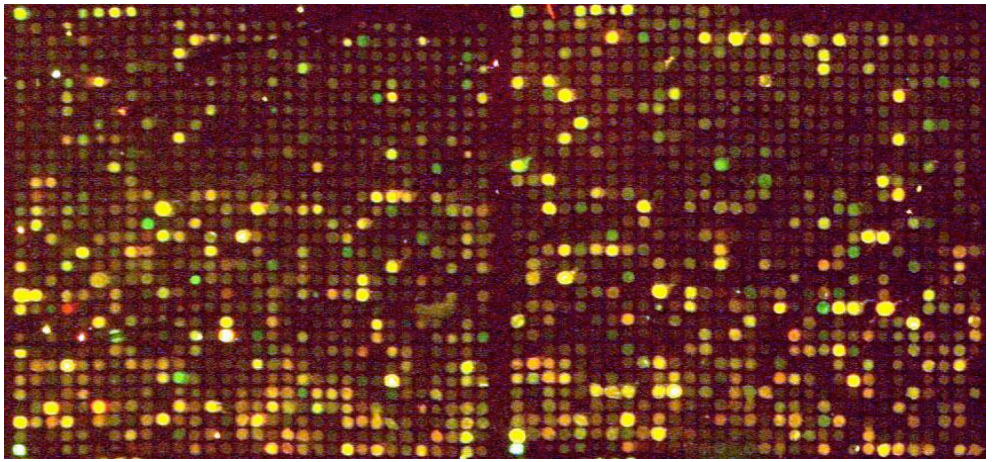
expressed in mutant



“FlyArray, Heidelberg”

Genome annotation, gene prediction, primer calculation, PCR, and spotting were done by the FlyArray consortium Heidelberg (Groups of J. Hoheisel, R. Paro, F. Sauer)

High density array with 47.616 spots: 21.306 double spotted ORFs plus 2502 controls,

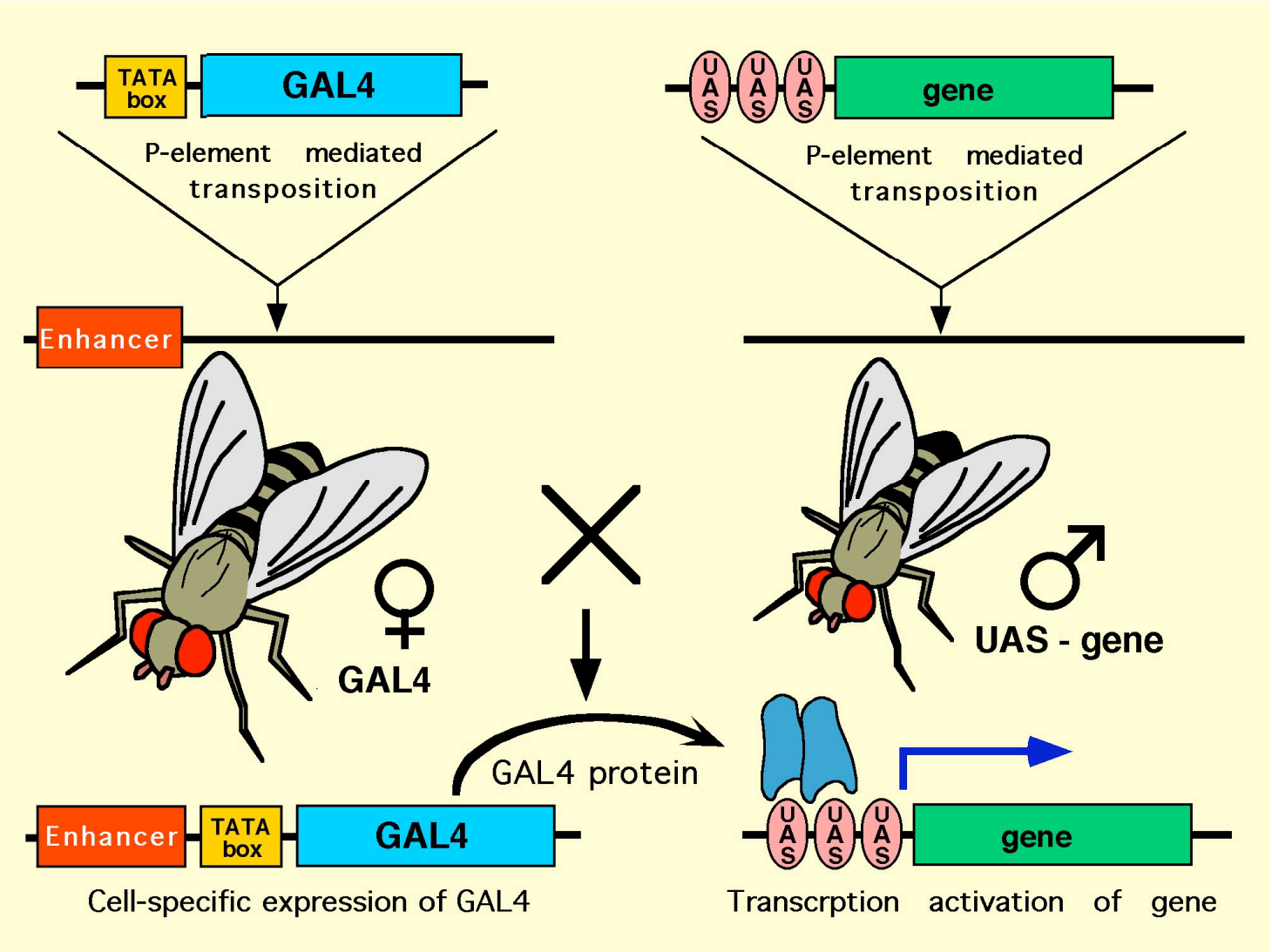


Aim: Identification of *gcm*-dependent target genes

- **Genome-wide search based on microarray-experiments**

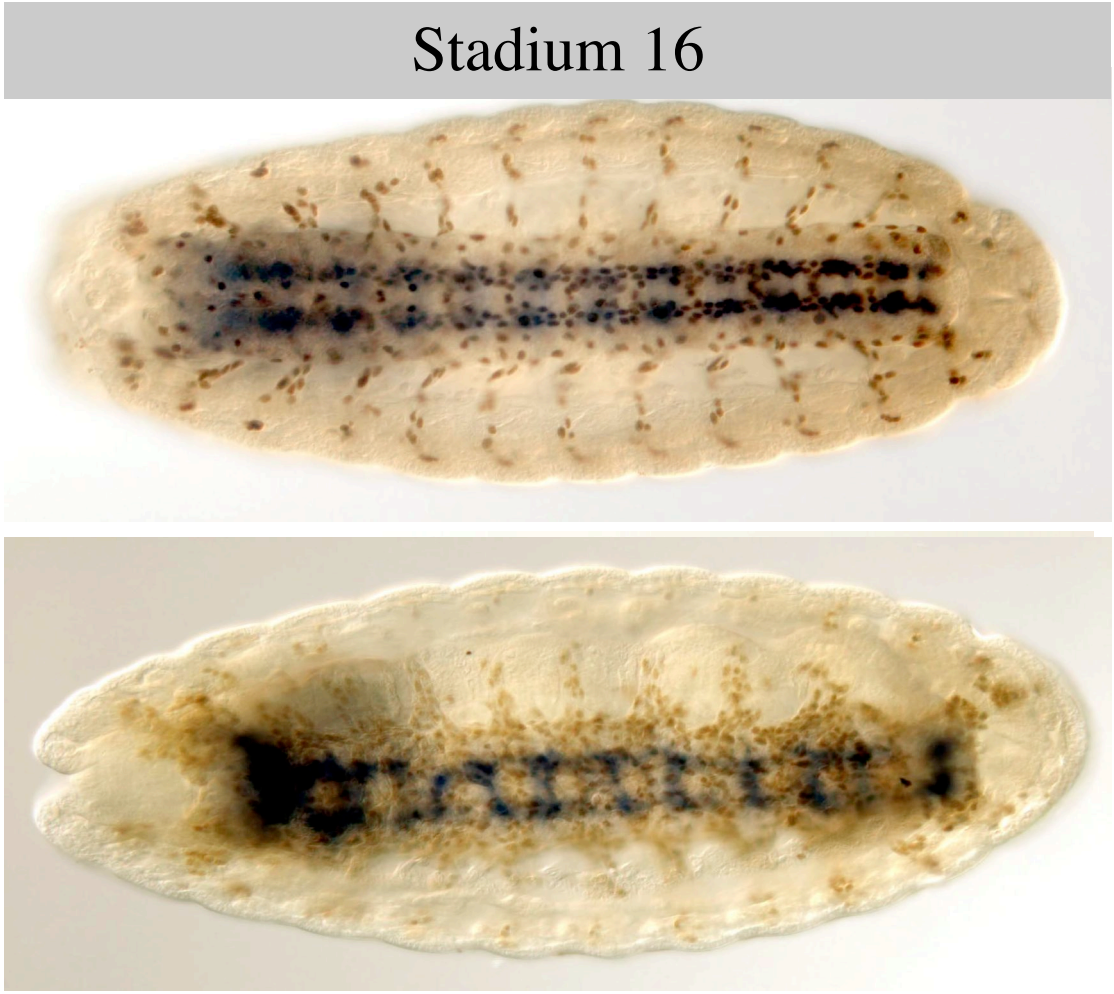
- **two approaches :**
  - **ectopic expression of *gcm* with the UAS/Gal4 System (gain-of-function/GOF)**

The UAS/GAL4 System



Brandt and Perrimon, 1993

Ektopic expression of *gcm* transforms potential neurons into glial cells

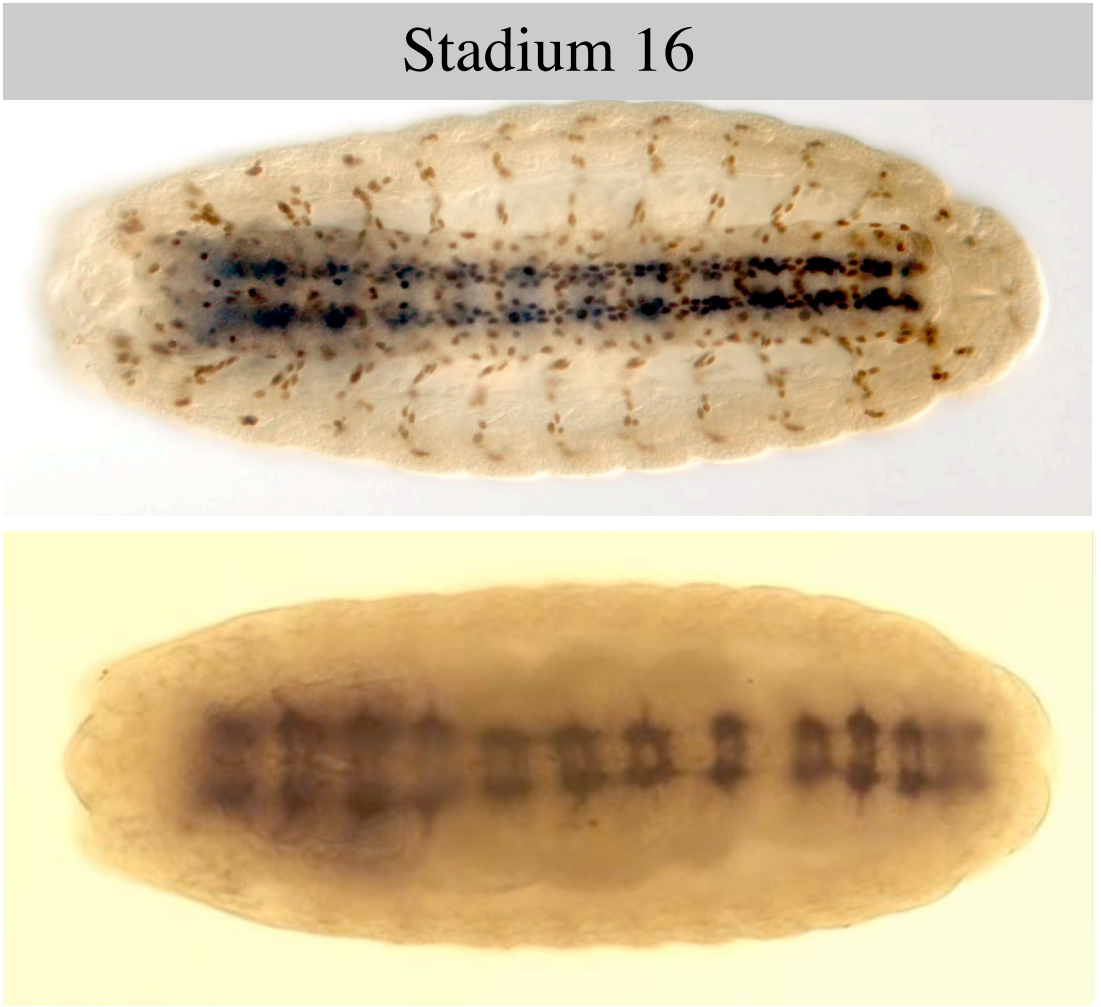


Aim: Identification of *gcm*-dependent target genes

- **Genome-wide search based on microarray-experiments**

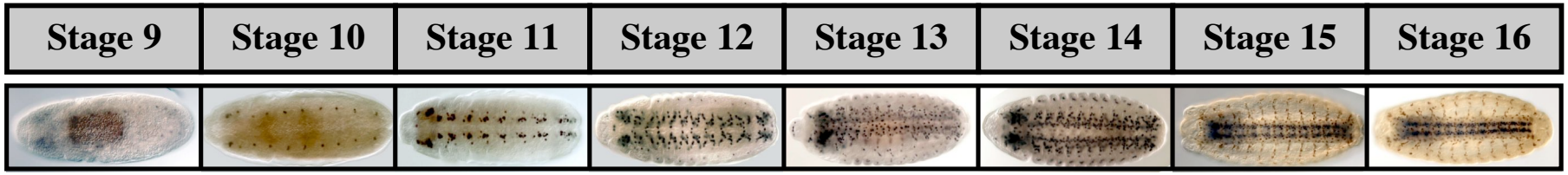
- **two approaches :**
  - **ectopic expression of *gcm* with the UAS/Gal4 System (gain-of-function/GOF)**
  - **loss-of-function of *gcm*(LOF)**

Gcm loss-of-function embryos lack nearly all glial cells



Microarray time course approach

wt



Checklist for quality controls and replicates before microarray evaluation

- Check developmental and genetic background (Antibody-staining) before RNA-Isolation
- Check degradation of Total-RNA (RNA-Gel)
- include dye-swaps (Cy3/Cy5)
- at least 4 replicates for each stage (double-spotted arrays)

Evaluation and result of the microarray approach

Quality control and determination of the methodical and genetic background

- determination of significantly differentially regulated genes
- equitation of non-equal genetic backgrounds by a „pooled“ wildtype control (GOF only)

→ reliable and more sensitive evaluation of data

Use of microarray-software

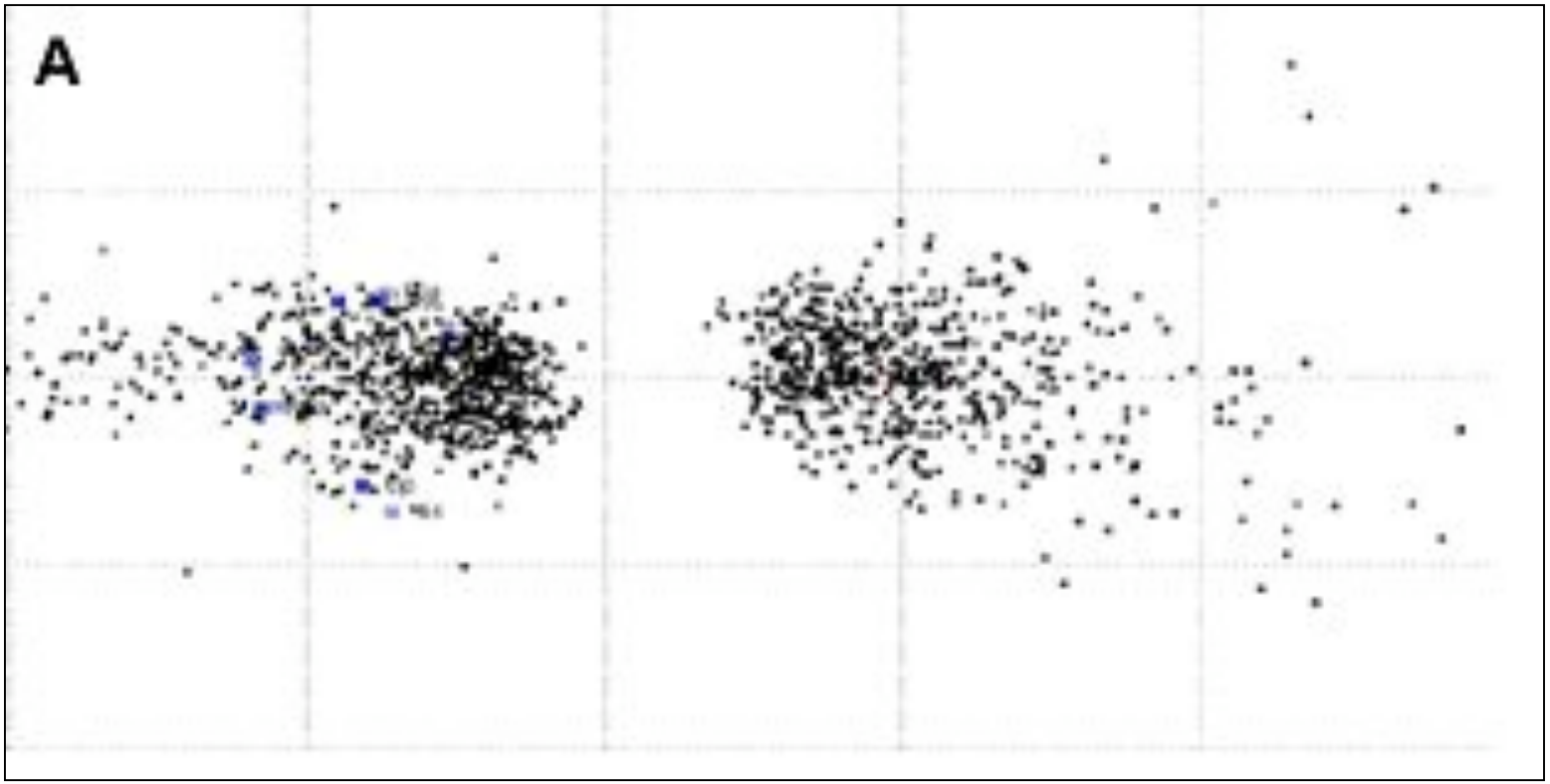
- Genepix as „spotfinder“: extraction of raw data
- MChips-Software:

  - background subtraction
  - normalization
  - determination of correlation coefficient: comparison of replicates on the array

  filtering by intensity: at least one value above threshold
  filtering by ratio 1.5: at least one value above threshold
  filtering by reproducibility
  correspondance analysis (CA)

CA-Plot

graphical illustration to see comparability of your replicated arrays and the difference between your data (wt:mutant)



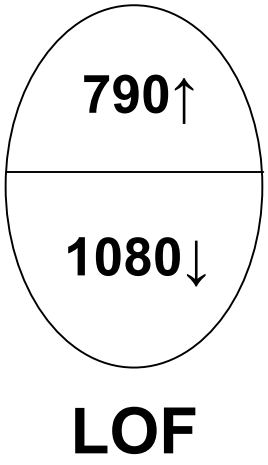
Problem: Still too many candidate genes

GOF	St. 9	St. 10	St. 11	St. 12	St. 13	St. 14	St. 15	St. 16	Total No.
No. of diff. reg. genes (total)	877	743	425	395	388	107	107	111	**1413**
Upregultated genes (>1.5)	399	496	328	372	322	98	106	89	**789**
Downregulated genes (<-1.5)	478	247	97	23	66	9	1	22	**625**

LOF	St. 9	St. 10	St. 11	St. 12	St. 13	St. 14	St. 15	St. 16	Total No.
No. of diff. reg. genes (total)		598	575	627	632	245		560	**2086**
Upregultated genes (>1.5)		353	193	180	269	161		386	**1056**
Downregulated genes (<-1.5)		245	382	447	363	84		174	**1080**

The advantage of time course experiments: Profiling

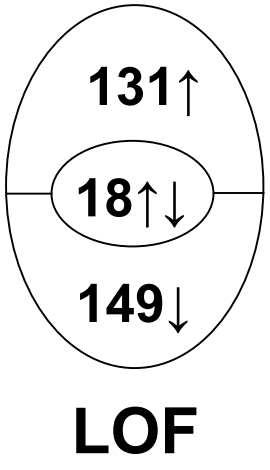
differentially regulated genes above ratio 1.5



Profiling

- expression profile in time course
- strength of expression
- comparison between GOF and LOF
- profile of potential „glial gene“

298 potential candidate genes

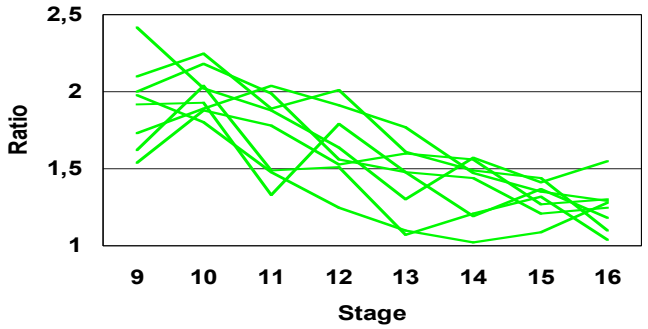


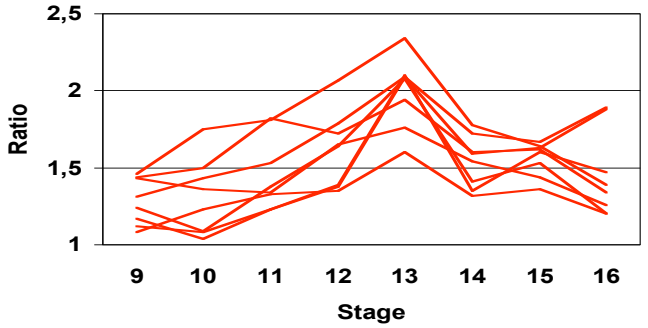
Manual „Profiling“ of candidate genes

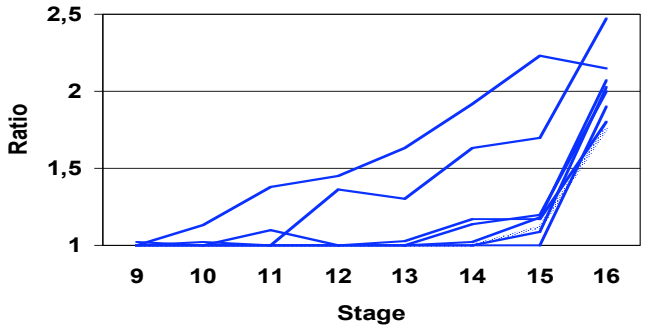
Gene	St. 10	St. 11	St. 12	St. 13	St. 14	St. 15	St. 16	ISH result, Literature
CG11164	+1.90	+2.17	+1.85	+2.28	+1.98	+1.81	+1.81	+1.89	ZNS ubiquitär
CG1962	+3.29	+3.03	+3.02	+3.20	+2.14	+2.28	+2.28	+1.93	ZNS, Glia + Neurone, BDGP
CG2051	+1.50	+1.52	+1.47	+1.69	+1.84	+1.50	+1.44	+1.44	ZNS ab 14 schwach (LG, EG)
CG16757	+1.73	+1.89	+2.04	+1.91	+1.77	+1.47	+1.35	+1.29	ML, ZNS Glia?
CG4523	+1.62	+1.82	+1.77	+1.84	+1.66	+1.48	+1.44	+1.37	ZNS, Glia?
Ptp10D	+1.62	+2.04	+1.49	+1.51	+1.07	+1.21	+1.32	+1.04	ZNS, Glia + Neurone
csw	+2.00	+2.18	+1.99	+1.56	+1.48	+1.44	+1.21	+1.25	ZNS ubiquitär
CG8983	+1.98	+1.80	+1.48	+1.25	-1.25	+1.02	+1.09	+1.28	ML
CG9796	+2.22	+2.38	+1.49	+1.09	-1.47	-1.29	-1.02	+1.05	tendon cells
CG7433	+1.47	+2.14	+2.45	+2.78	+1.97	+2.00	+1.87	+2.07	BDGP, schwach in LG
gcm	+1.03	+1.60	+1.51	+2.36	+2.54	+2.57	+2.41	+2.97	
CG1677	+1.43	+1.62	+1.59	+1.80	+1.90	+1.46	+1.51	+1.56	ZNS ubiquitär, evtl EG
Cam	+1.44	+1.50	+1.82	+1.72	+1.94	+1.60	+1.62	+1.34	ZNS ubiquitär, PNS einzelne Zellen
UbcD2	+1.37	+1.51	+1.51	+1.59	+1.94	+1.47	+1.56	+1.38	ZNS ubiquitär, nicht PNS
smid	+1.41	+1.56	+1.70	+1.89	+1.43	+1.46	+1.46	+1.38	ZNS ubiquitär
CG10326	+1.49	+1.60	+1.37	+1.79	+1.47	+1.26	+1.42	+1.21	ZNS, ubiquitär
CG8171	+1.31	+1.43	+1.53	+1.79	+2.09	+1.72	+1.67	+1.89	ZNS, Glia + Neurone
CG6218	+1.00	+1.13	+1.43	+1.78	+1.63	+1.92	+2.23	+2.15	CBG
RpII 140	+1.09	+1.48	+1.43	+1.58	+2.06	+1.68	+1.37	+1.53	ubiquitär, im ZNS in einzelnen Zellen (G+N)
CG3227	+1.24	+1.09	+1.38	+1.64	+2.08	+1.41	+1.53	+1.20	BDGP, LG, EG, PG
glu	+1.43	+1.36	+1.34	+1.65	+1.76	+1.54	+1.44	+1.26	ZNS ubiquitär, später LG, EG, +, Egger
Uba2	+1.15	+1.40	+1.39	+1.51	+1.89	+1.40	+1.47	+1.26	ZNS ubiquitär
BcDNA:LD08534	+1.17	+1.04	+1.23	+1.38	+2.09	+1.59	+1.63	+1.88	ZNS Glia + Neurone, Egger
CG15141	+1.43	+1.31	+1.29	+1.46	+1.60	+1.47	+1.64	+1.51	ZNS Neurone + Glia?
Map60	+1.12	+1.08	+1.23	+1.39	+2.10	+1.35	+1.60	+1.47	BDGP, ZNS ubiquitär, evtl. EG, LG
CG4266	+1.07	+1.30	+1.27	+1.36	+1.70	+1.21	+1.18	+1.26	ZNS ubiquitär
CG4936	+1.07	+1.08	+1.12	+1.25	+1.91	+1.10	+1.24	-1.06	ZNS Glia + Neurone
CG6418	+1.08	+1.23	+1.33	+1.35	+1.60	+1.32	+1.36	+1.20	LG, EG, SPG?, CG?, Neurone
pnut	+1.26	+1.31	+1.29	+1.23	+1.78	+1.27	+1.35	+1.44	ZNS ubiquitär
CG1153	+1.12	-1.09	-1.07	-1.25	1.25	-1.12	-1.42	+1.68	tendon cells
CG15860	+1.00	+1.00	+1.01	+1.00	+1.03	+1.17	+1.17	+1.77	PNS, ZNS, SPG
CG2893	+1.05	+1.00	-1.05	+1.00	+1.02	+1.10	+1.17	+1.65	viele Glia!, BDGP
CG3168	+1.02	+1.00	-1.10	-1.08	-1.08	+1.02	+1.18	+2.07	BDGP, LG, SPG, CBG, PG, EG,
CG6783	+1.02	+1.02	-1.23	-1.08	-1.15	-1.11	+1.09	+2.03	Freeman, BDGP, CBG
CG9336	+1.00	+1.00	-1.07	-1.06	-1.07	-1.04	+1.12	+1.75	PG, CBG,ML, ZNS
EG:22E5.11	+1.04	+1.00	-1.03	+1.09	+1.10	+1.13	+1.20	+1.54	PG, SPG, CBG, CG, EG

Profiling of temporal regulation

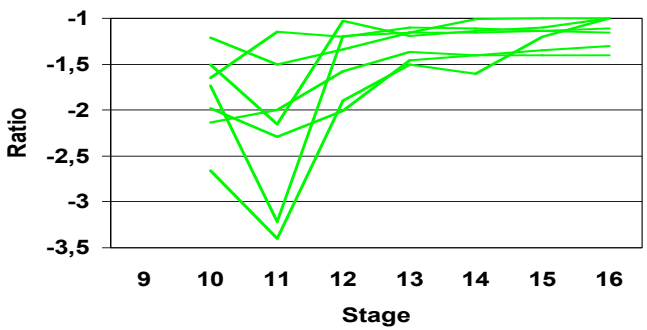
gain-of-function

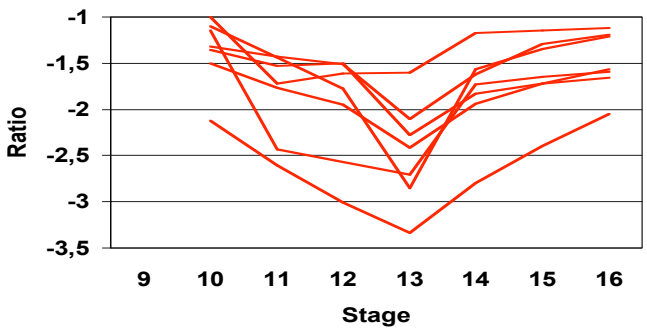


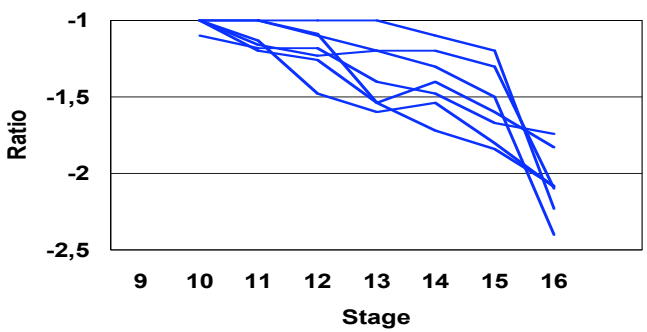




loss-of-function





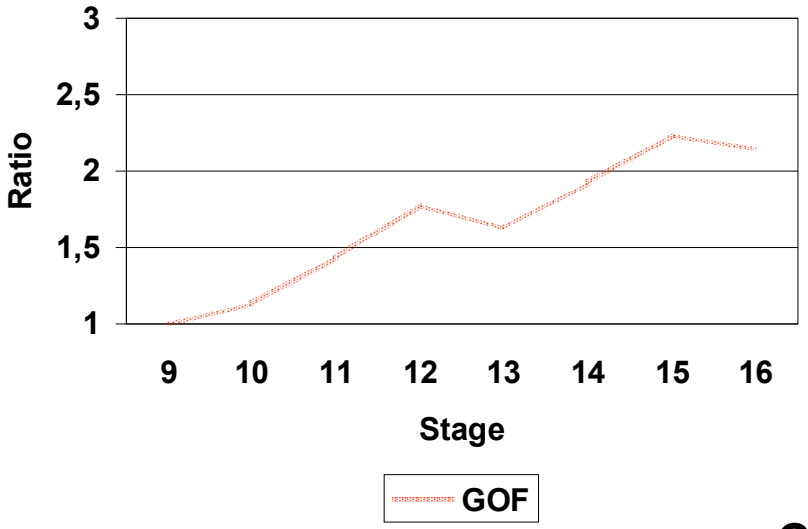


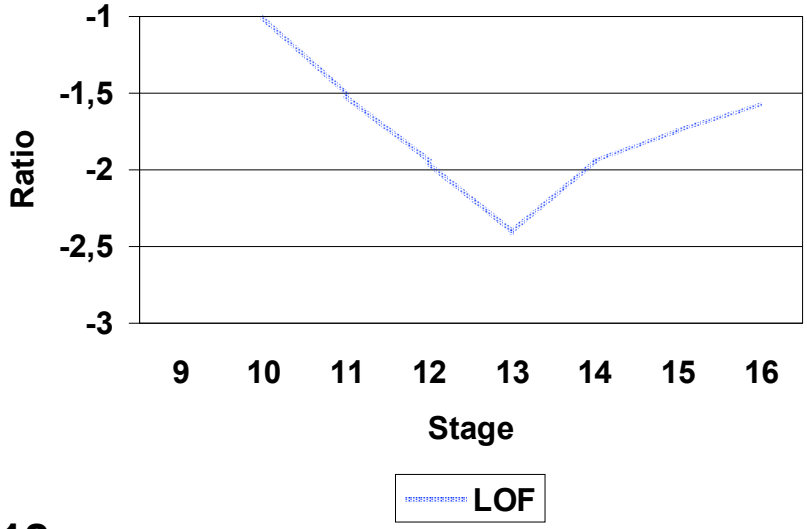
early regulated genes

mid regulated genes

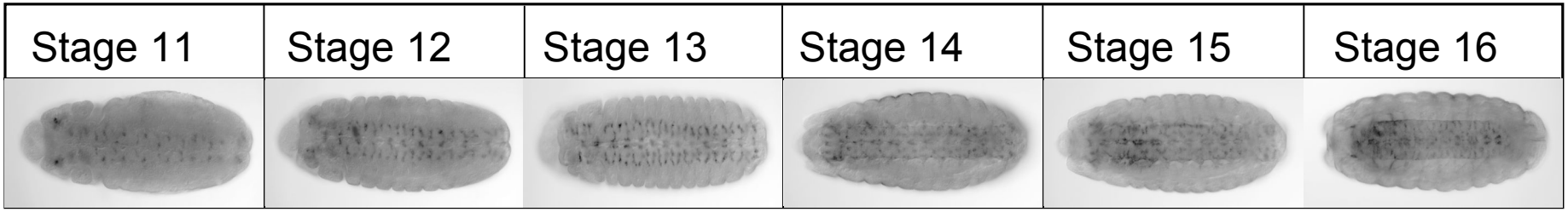
late regulated genes

Array-Profile versus *in vivo*





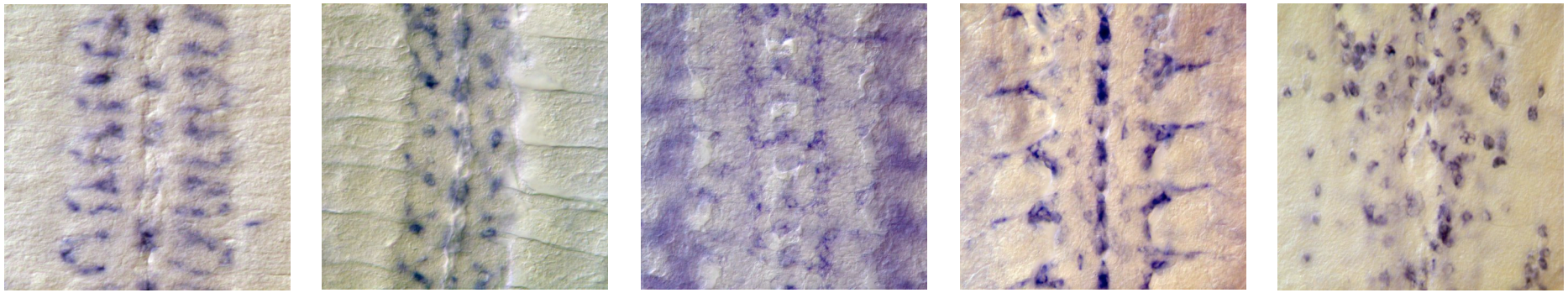
CG6218



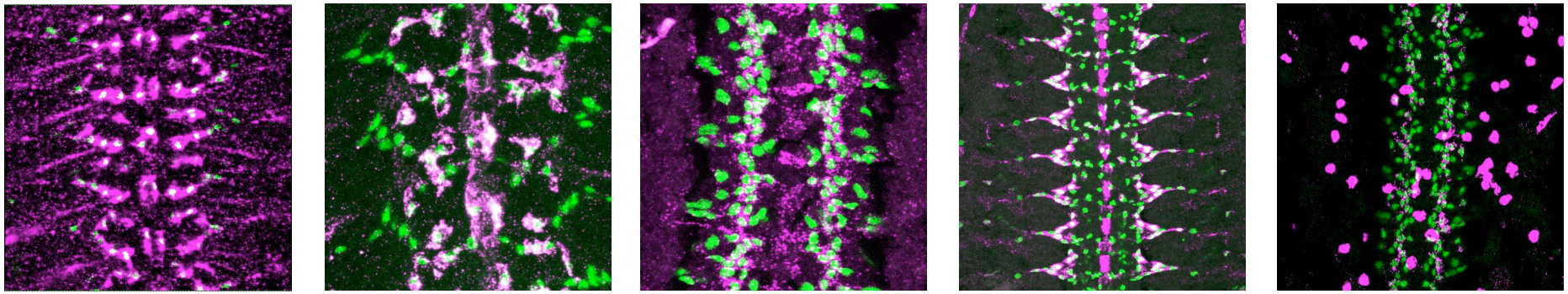
wt ISH

Are the filtered candidate genes expressed in glial cells?

HFA15994 HFA16115 HFA03125 HFA02311 HFA12894

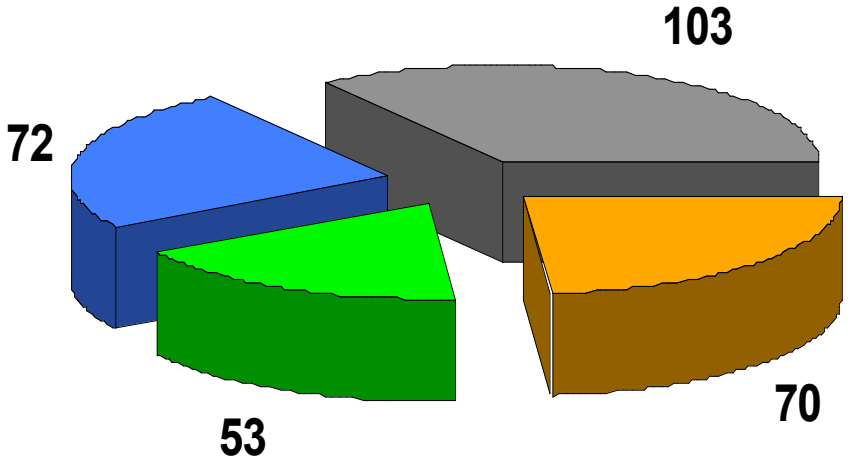


wt ISH, stage 14



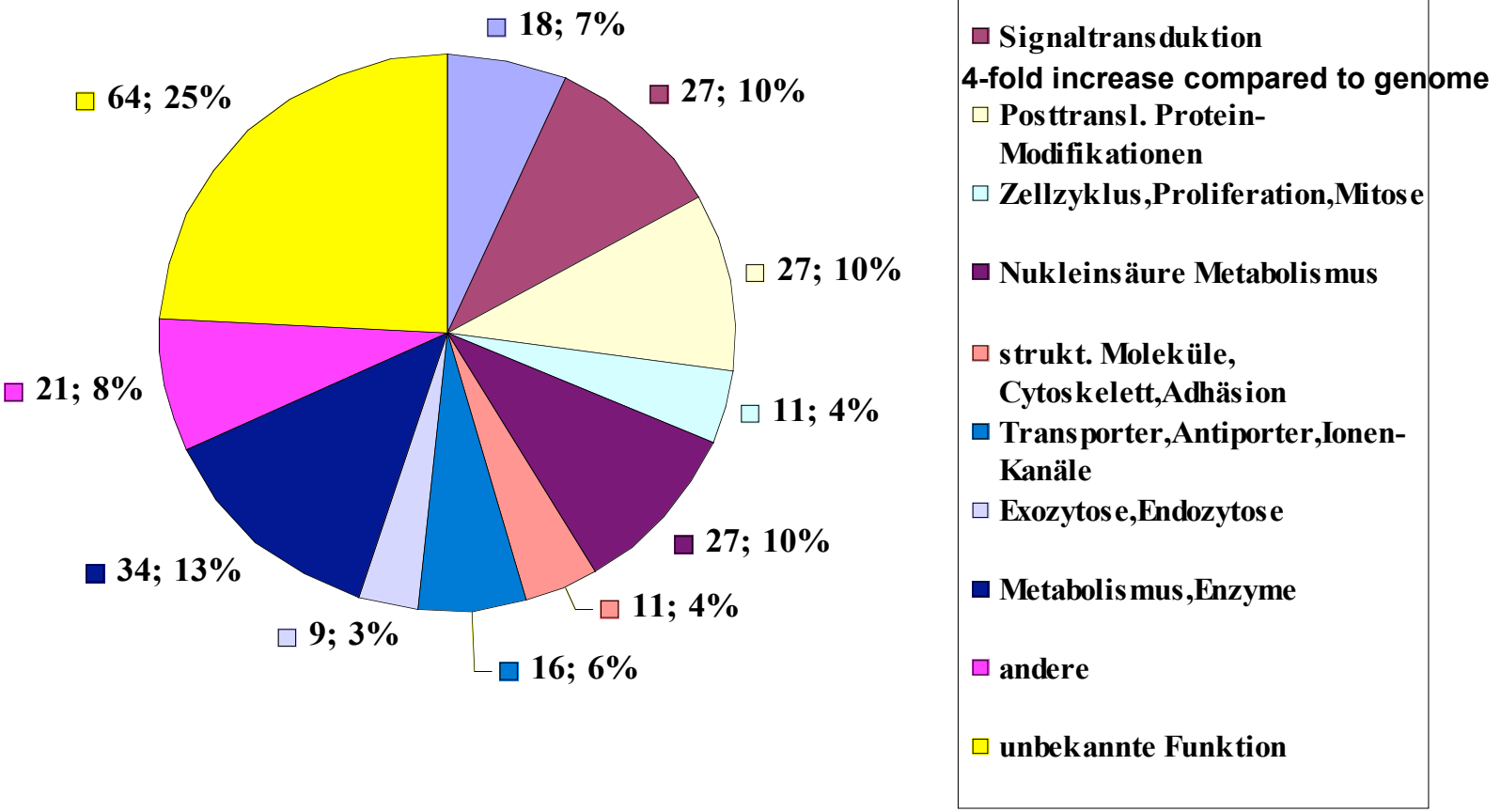
wt FISH α-Repo, stage 14, anterior

Potential candidate genes tested



14 exclusively expressed in glia
12 expressed in glia and neurons
26 ubiquitously in CNS and PNS
10 expressed in hematopoetic lineage
8 expressed in muscle tendon cells

novel *gcm* targets
known *gcm* targets
non-neuronal expression
unconfirmed results

Further ways to explore the data

- filtering for GO-Annotation
- filtering for pathways