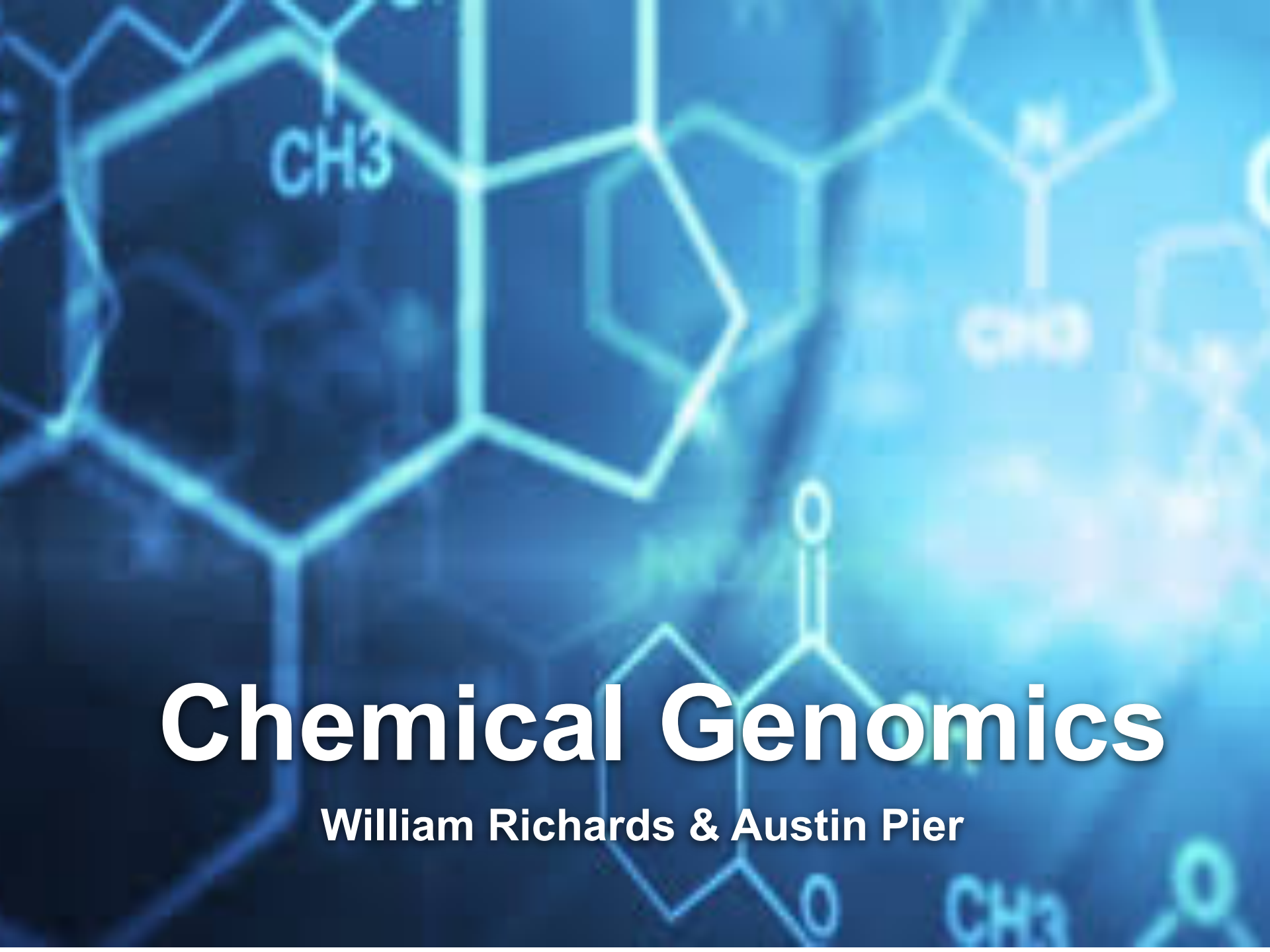
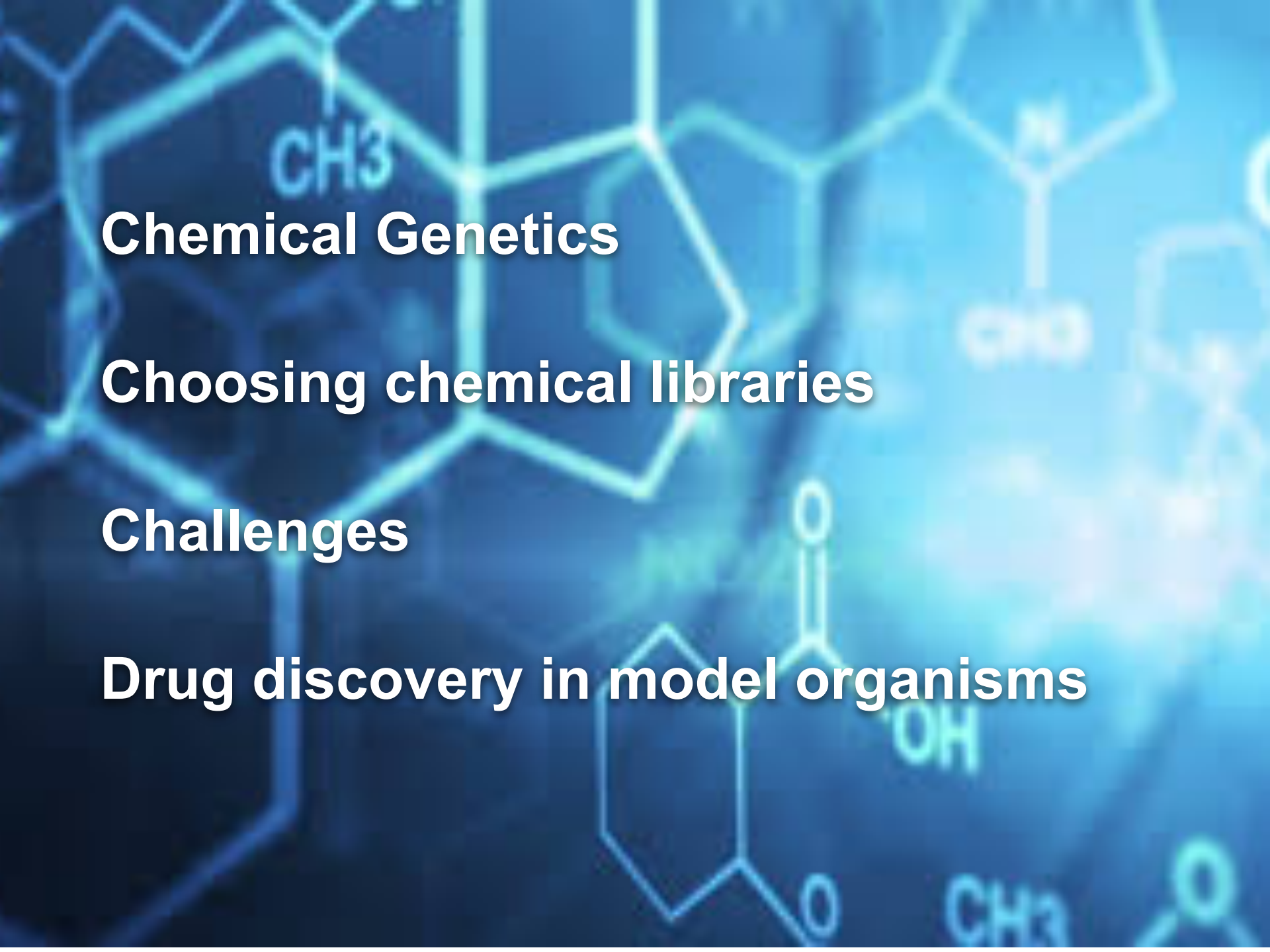
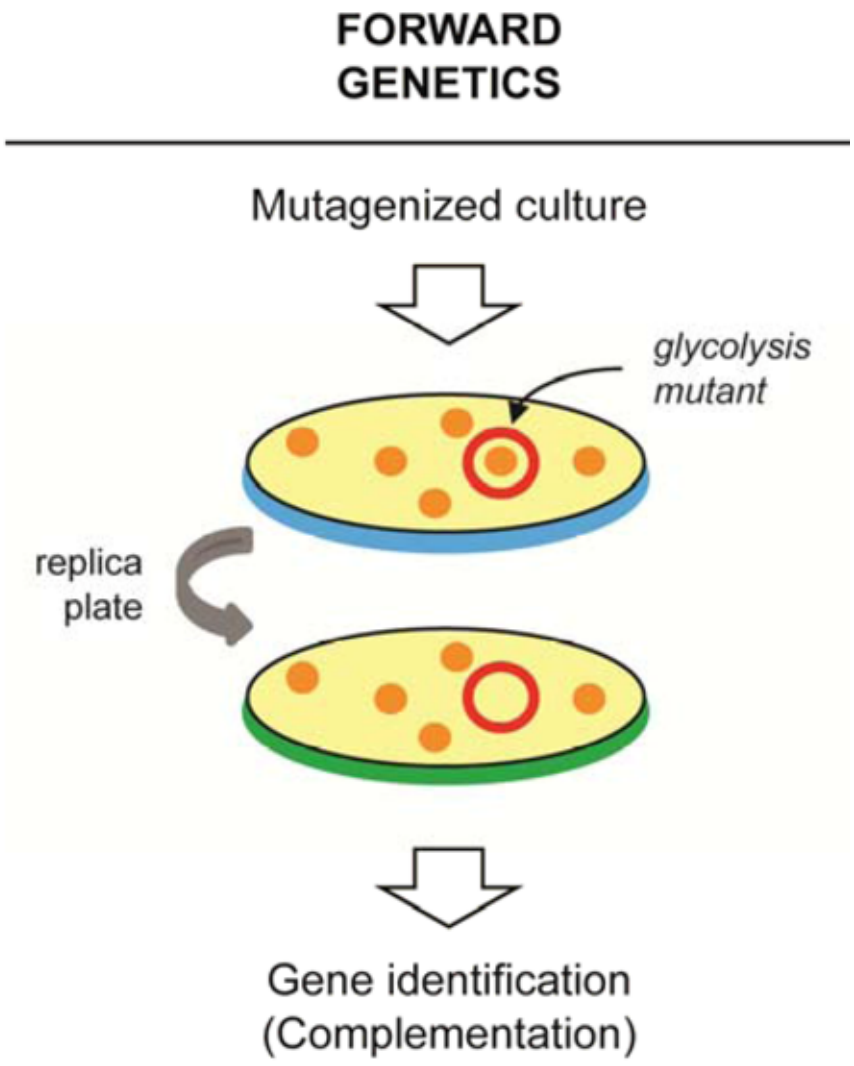


Chemical Genomics

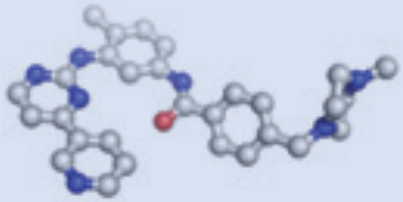
William Richards & Austin Pier


Chemical Genetics
Choosing chemical libraries
Challenges
Drug discovery in model organisms

What is chemical genetics?

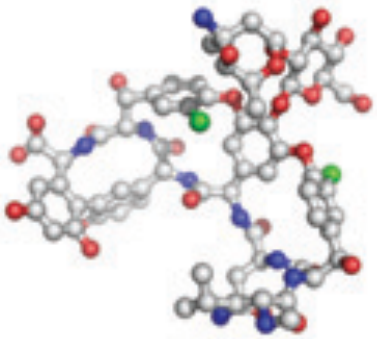


What are small molecules?



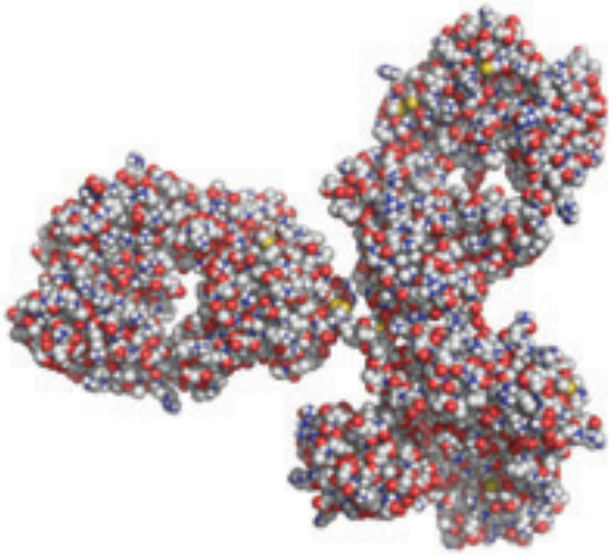
small molecule
(imatinib)

< 1000 Da



macrocycle
(vancomycin)

1-3 kDa

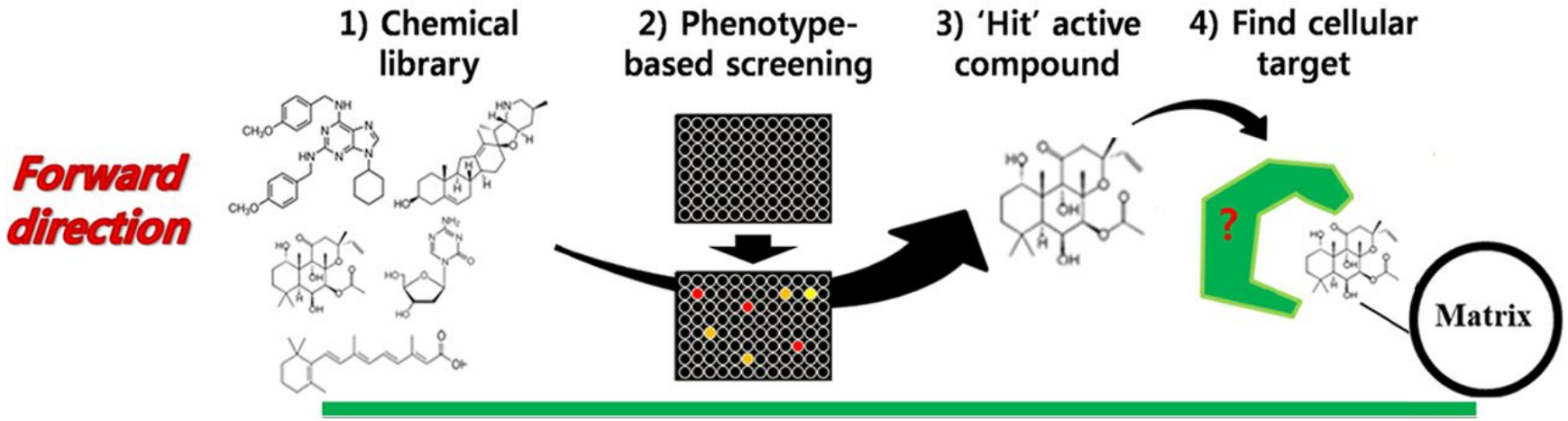


monoclonal antibody

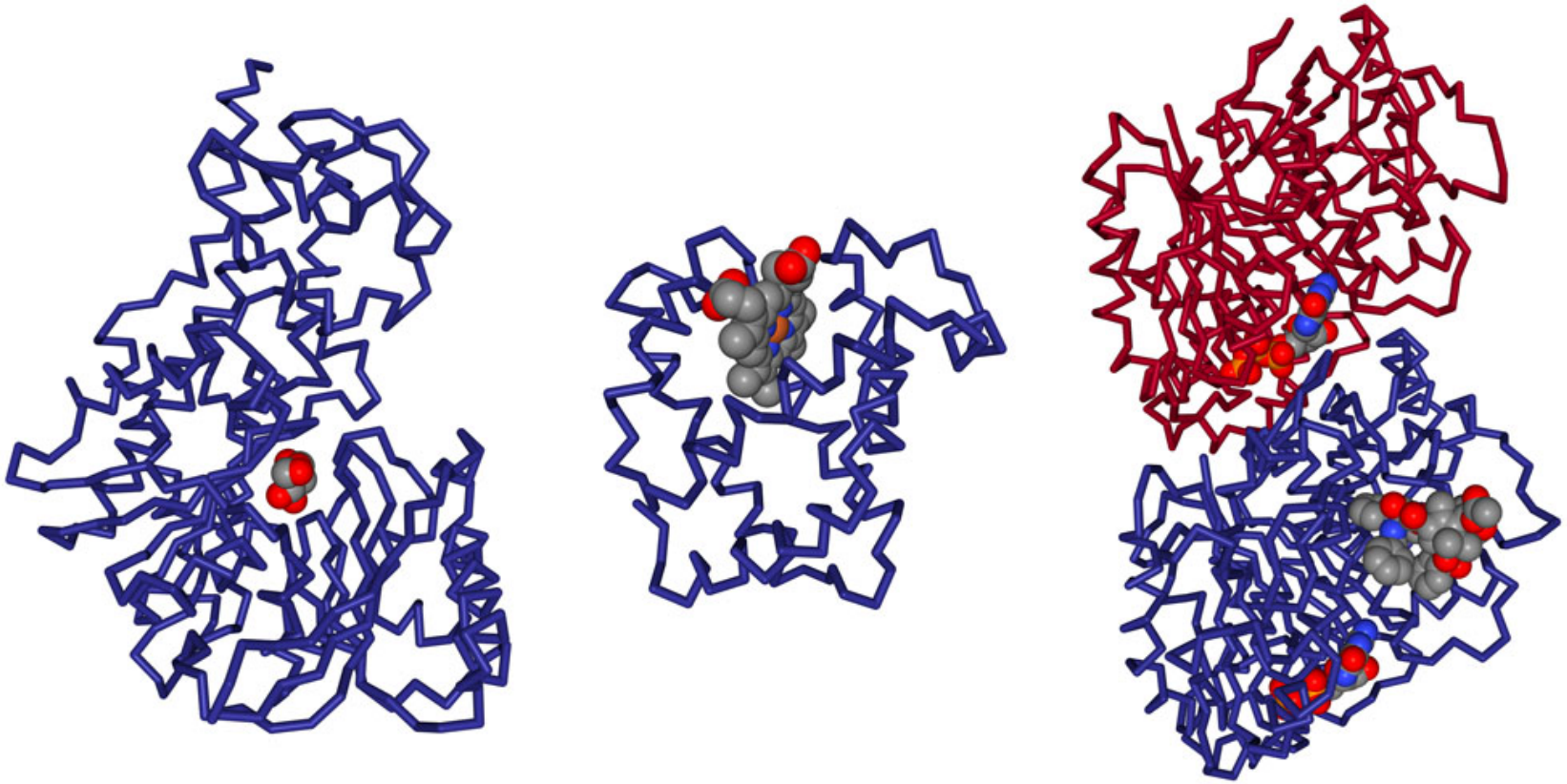
150 kDa

Advantage: Can target one function of multi-function protein

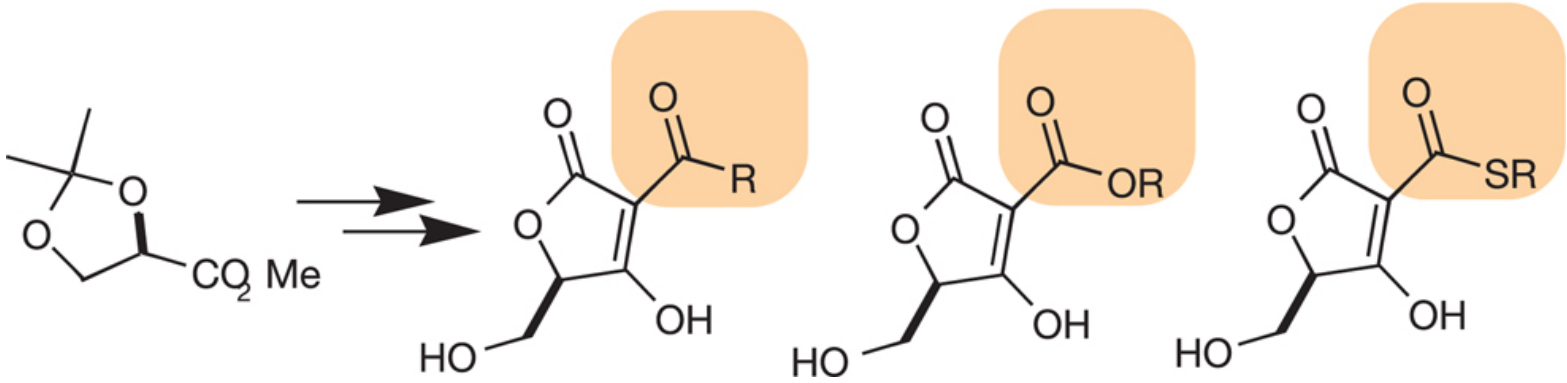
How do we use chemical genetics?



What makes an ideal chemical library?

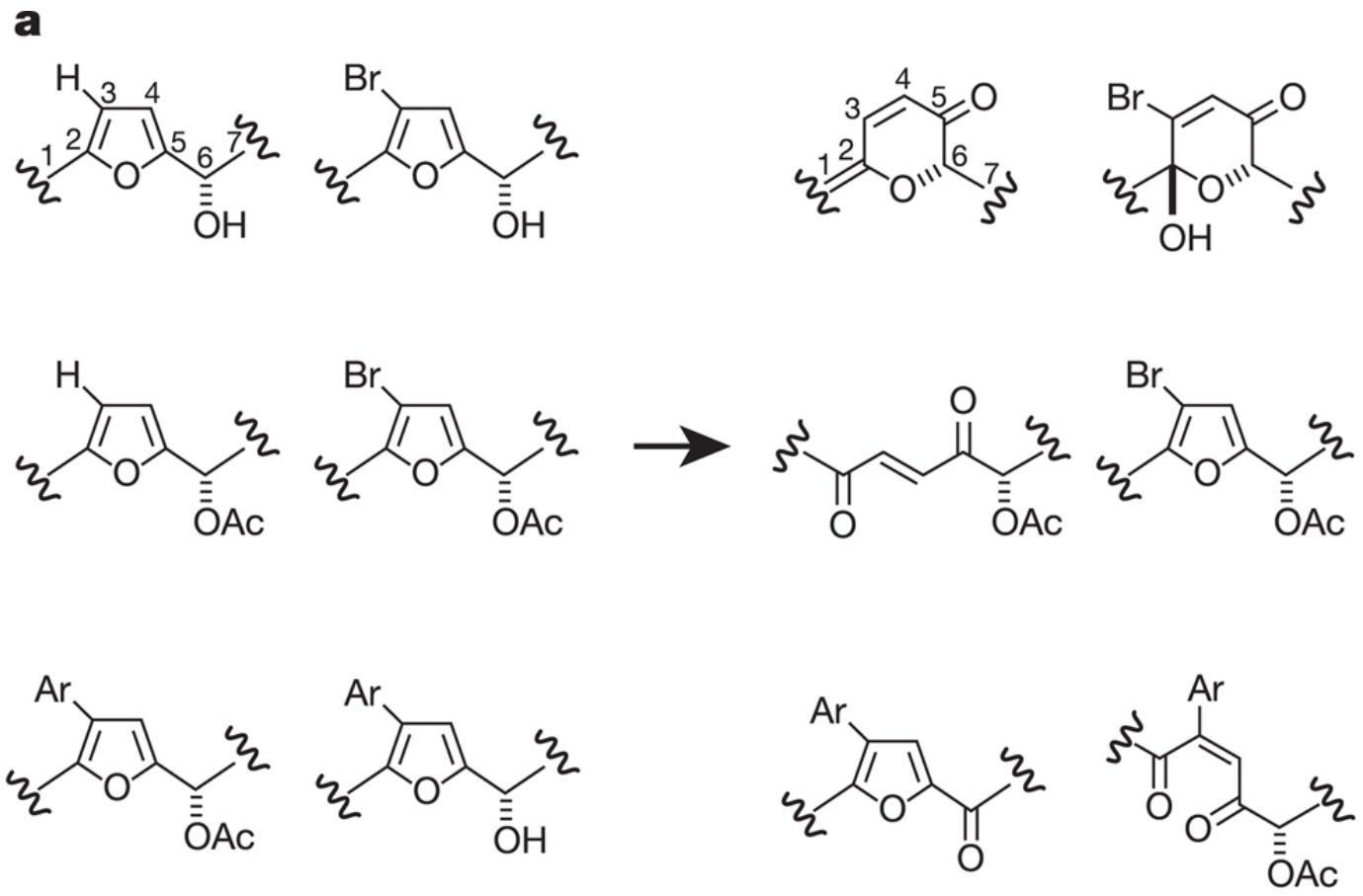


What are focused libraries?



Specific protein class target, Likely to be active

What are diversity-oriented libraries?

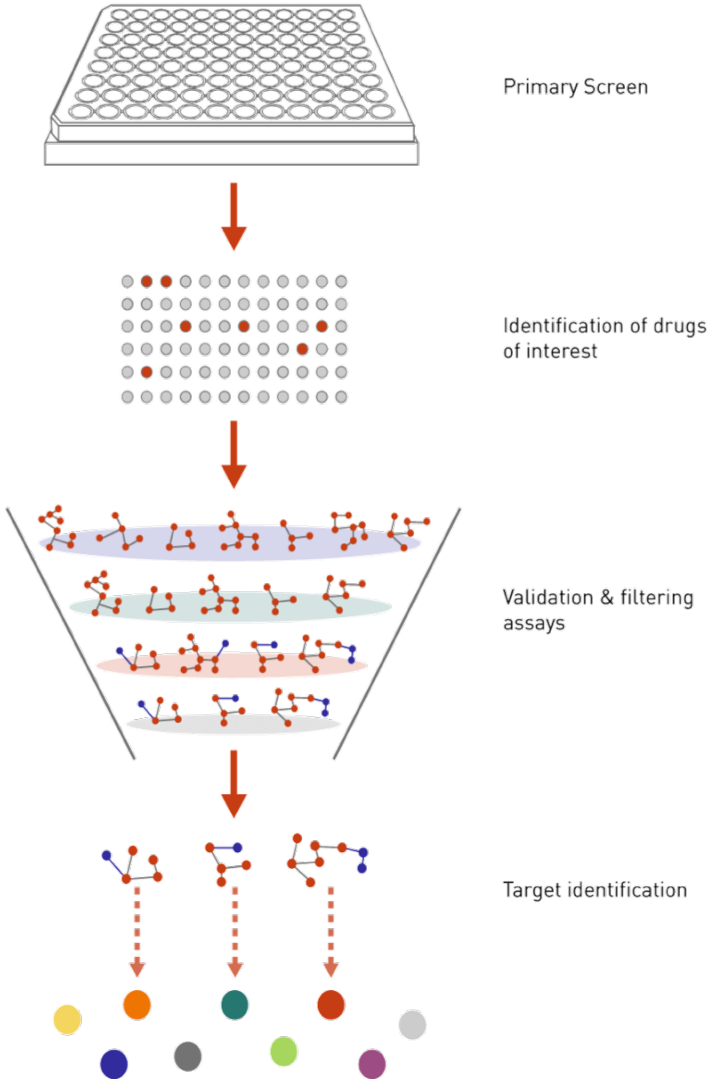


Broad screens
Target new protein classes
Low chance of being active

How are libraries analyzed?

Protein-binding Assay

Phenotypic Activity Assay



What are protein-binding assays?

Labelled vs. Non-labelled

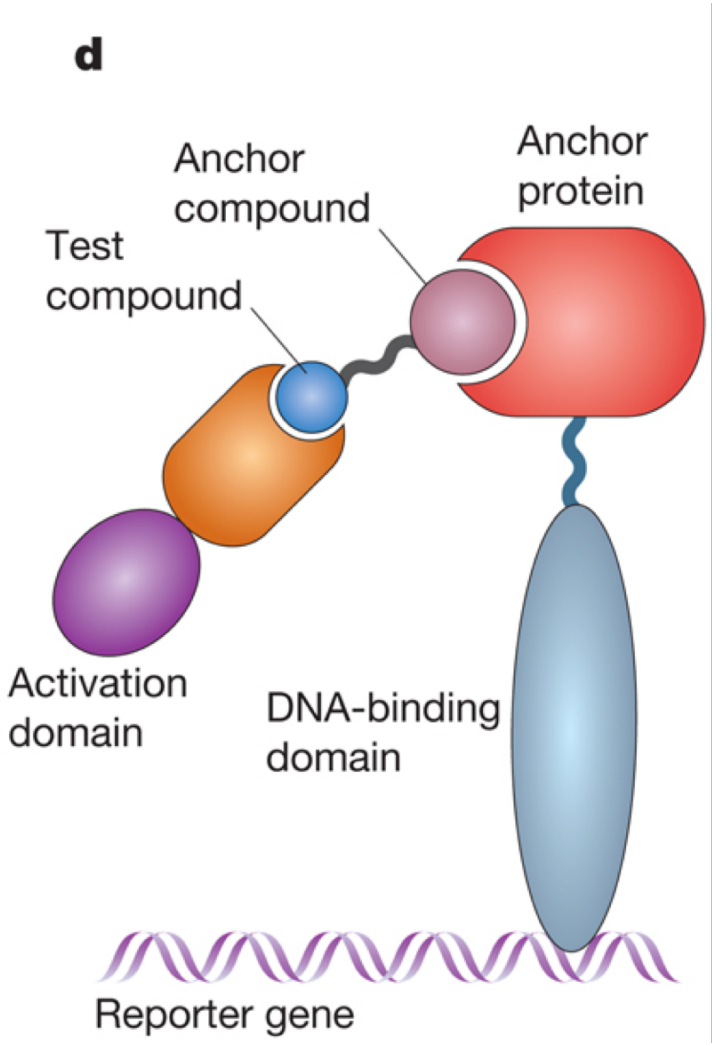
Label difficult to introduce

Use time and money

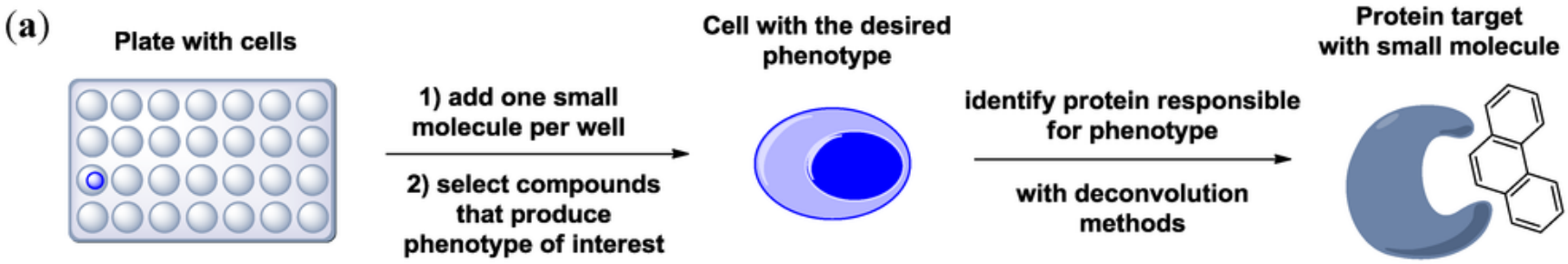
Need more protein

Slower

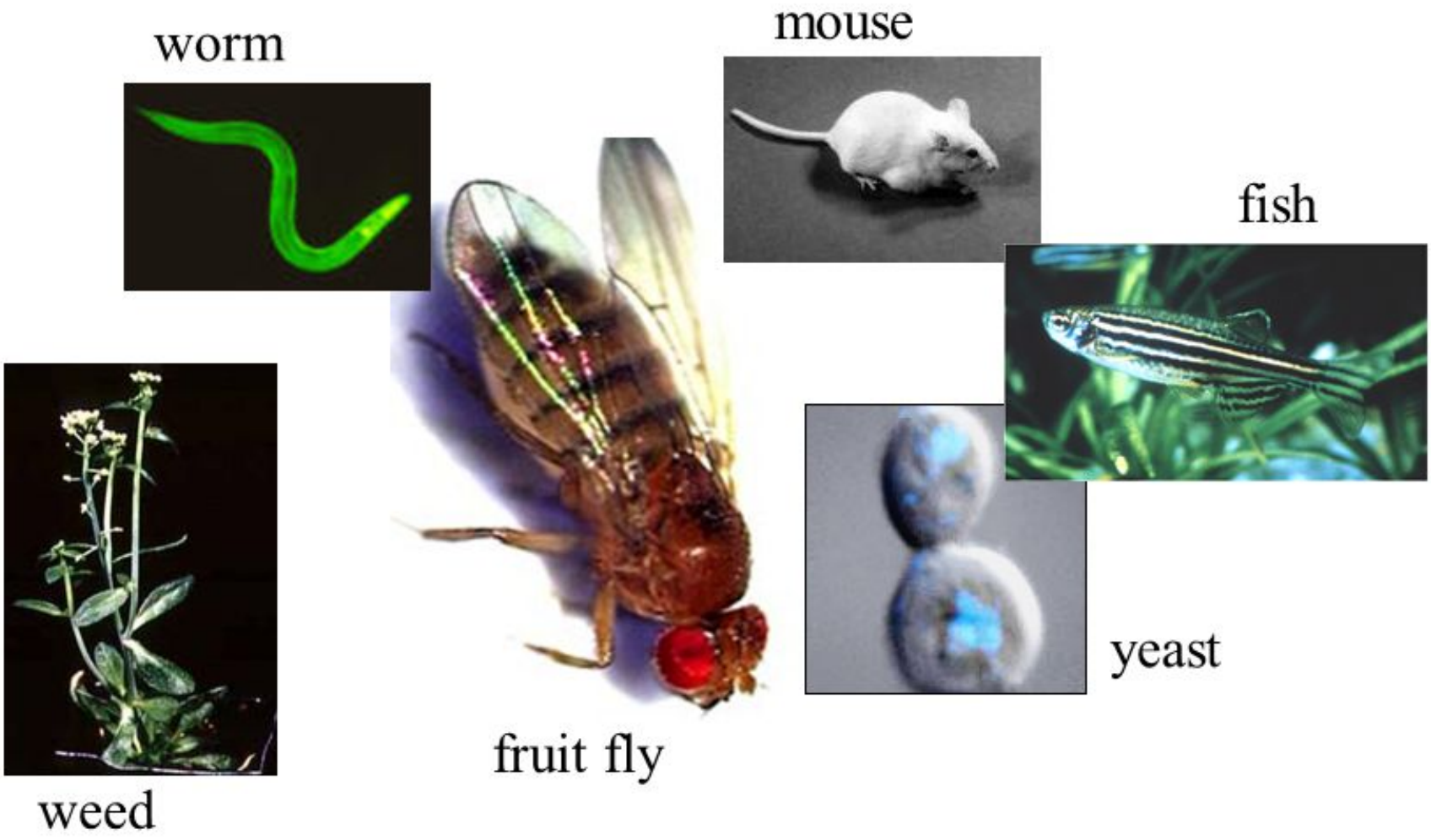
What is the three-hybrid system?



What is a phenotypic activity assay?

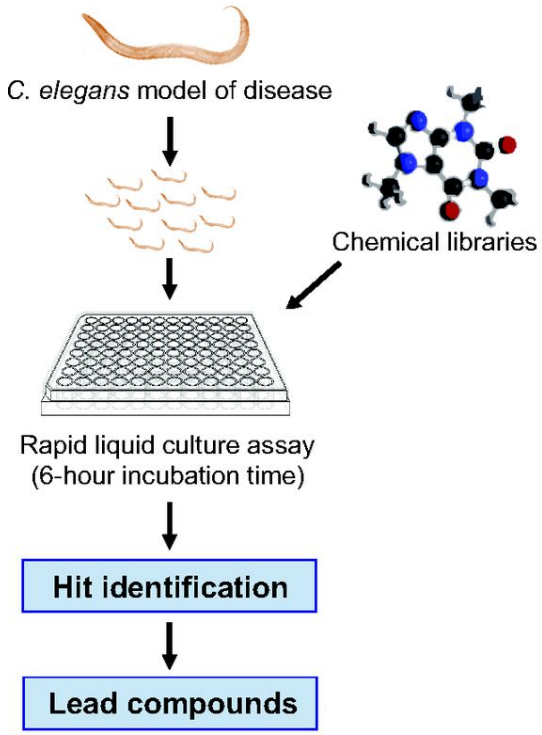


What model organism is useful in chemical genetics assays?



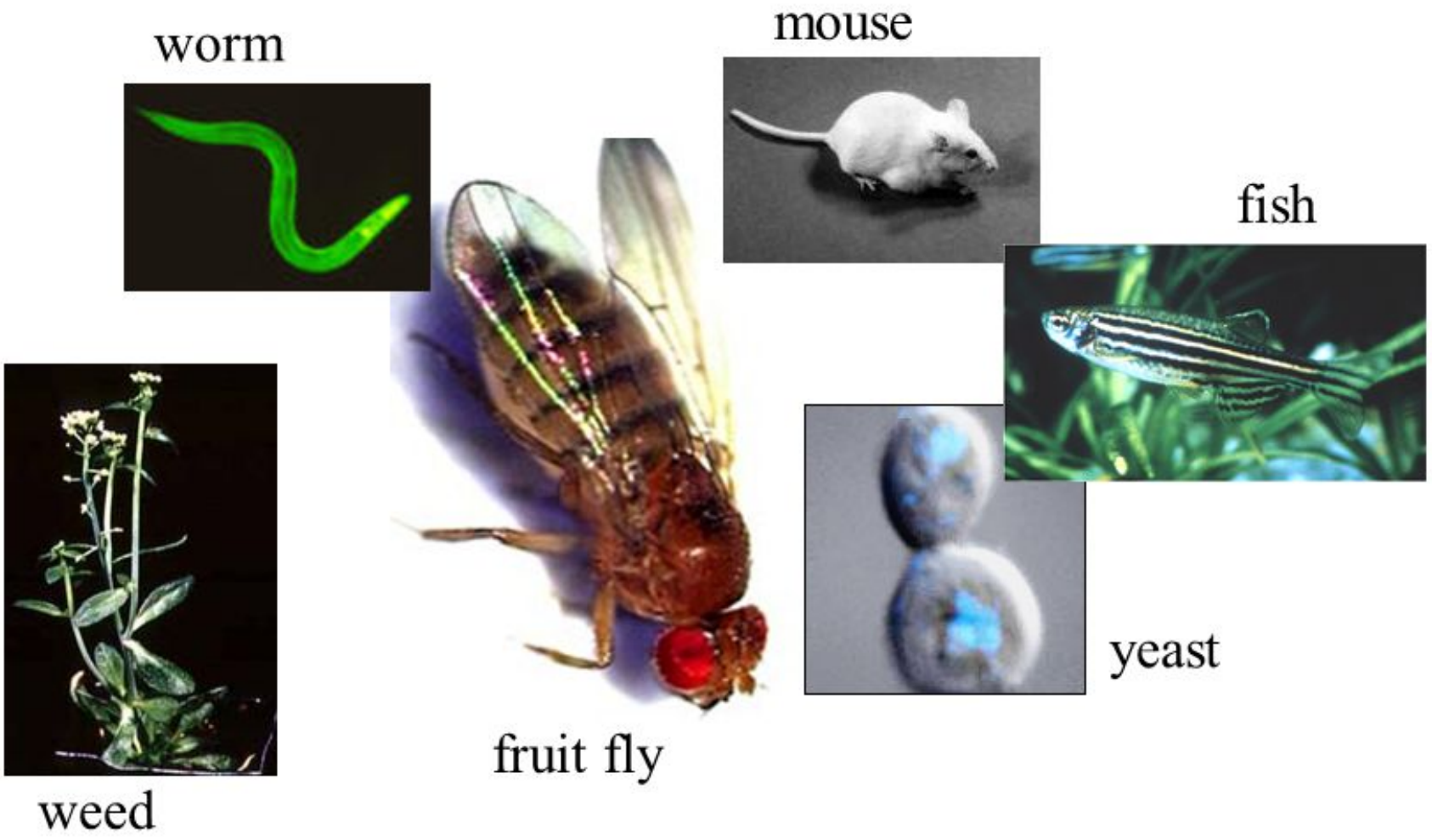
What are the best model organisms for performing chemical genomic assays?

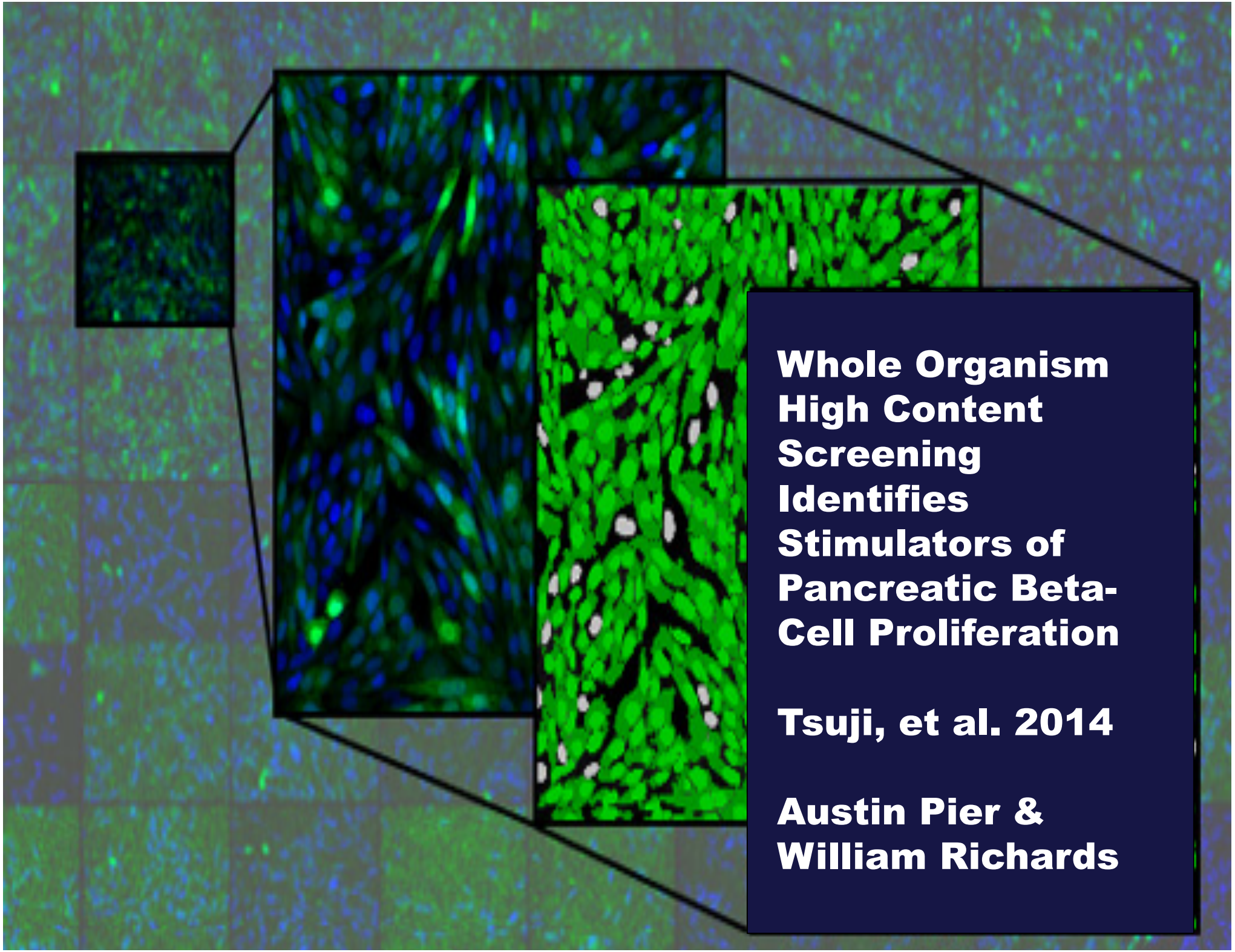
How is chemical genetics performed in model organisms?



Are mice good for chemical genetics?

Can you use chemical genetics with your model organism?





Whole Organism High Content Screening Identifies Stimulators of Pancreatic Beta-Cell Proliferation

Tsuji, et al. 2014

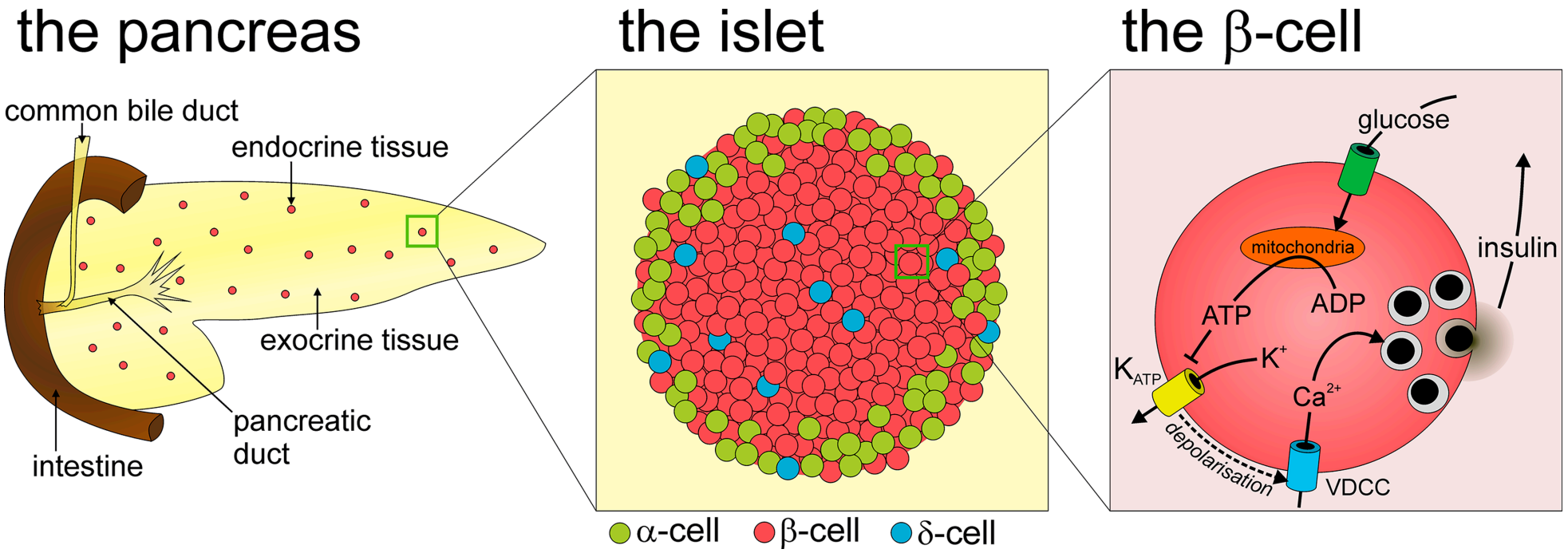
Austin Pier &
William Richards

What is Whole Organism High Content Screening?

What is Whole Organism High Content Screening?

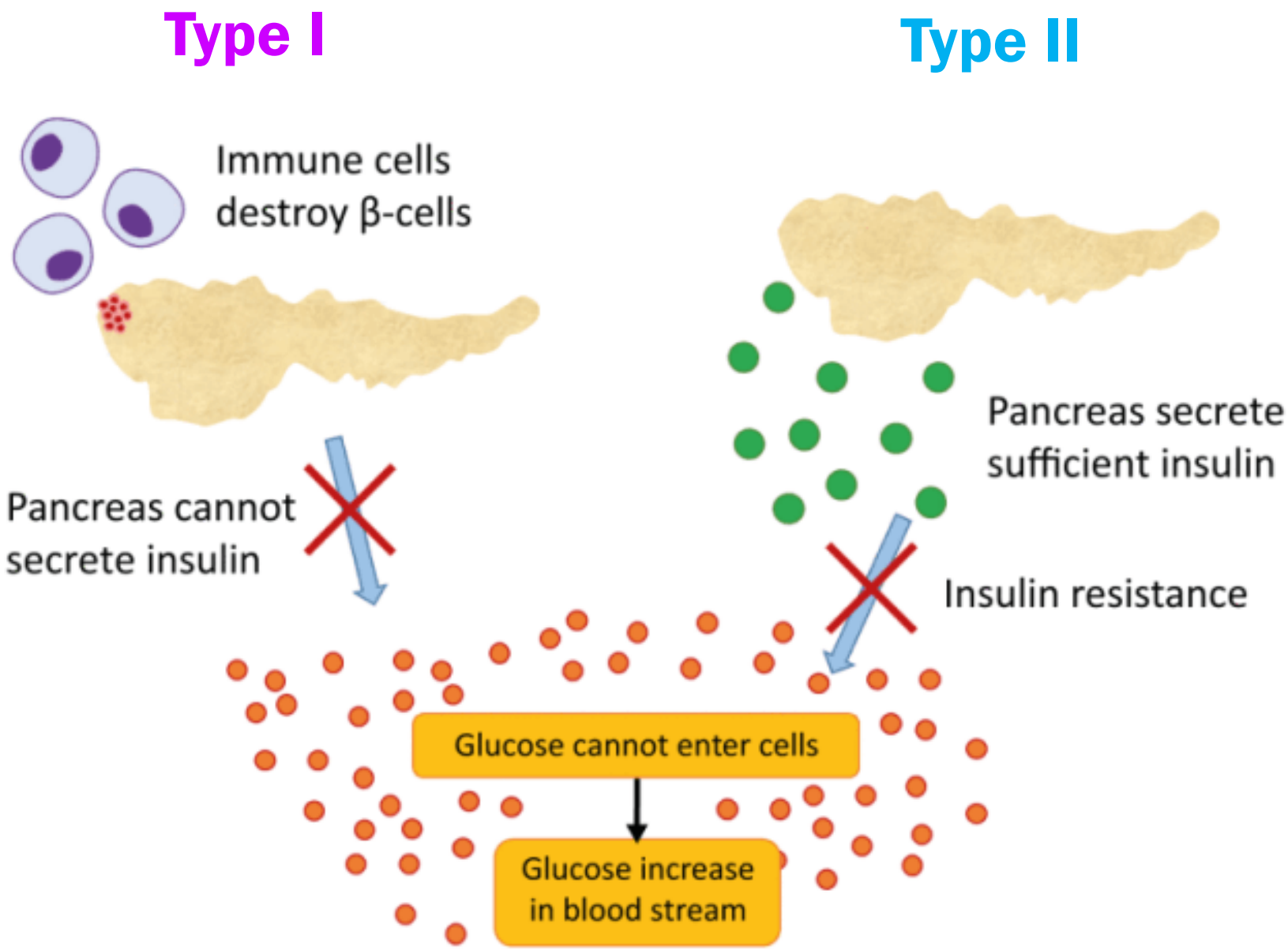
Visual and analytical method which utilizes microscopic fluorescent imaging to extract data from whole cell populations

What role do pancreatic beta-cells play in glucose uptake?

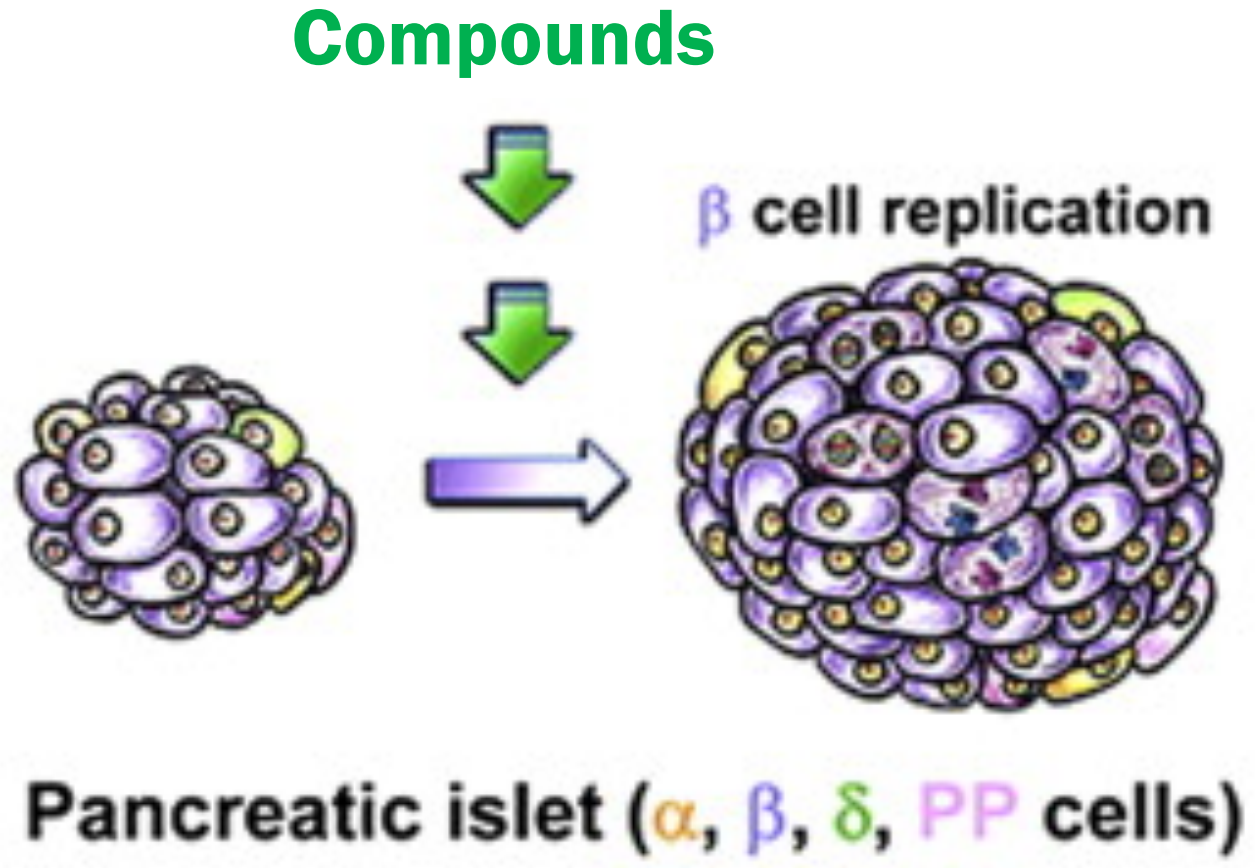


Pancreatic beta-cells produce insulin, which allows cells to utilize glucose in the blood.

What causes type I and type II diabetes?

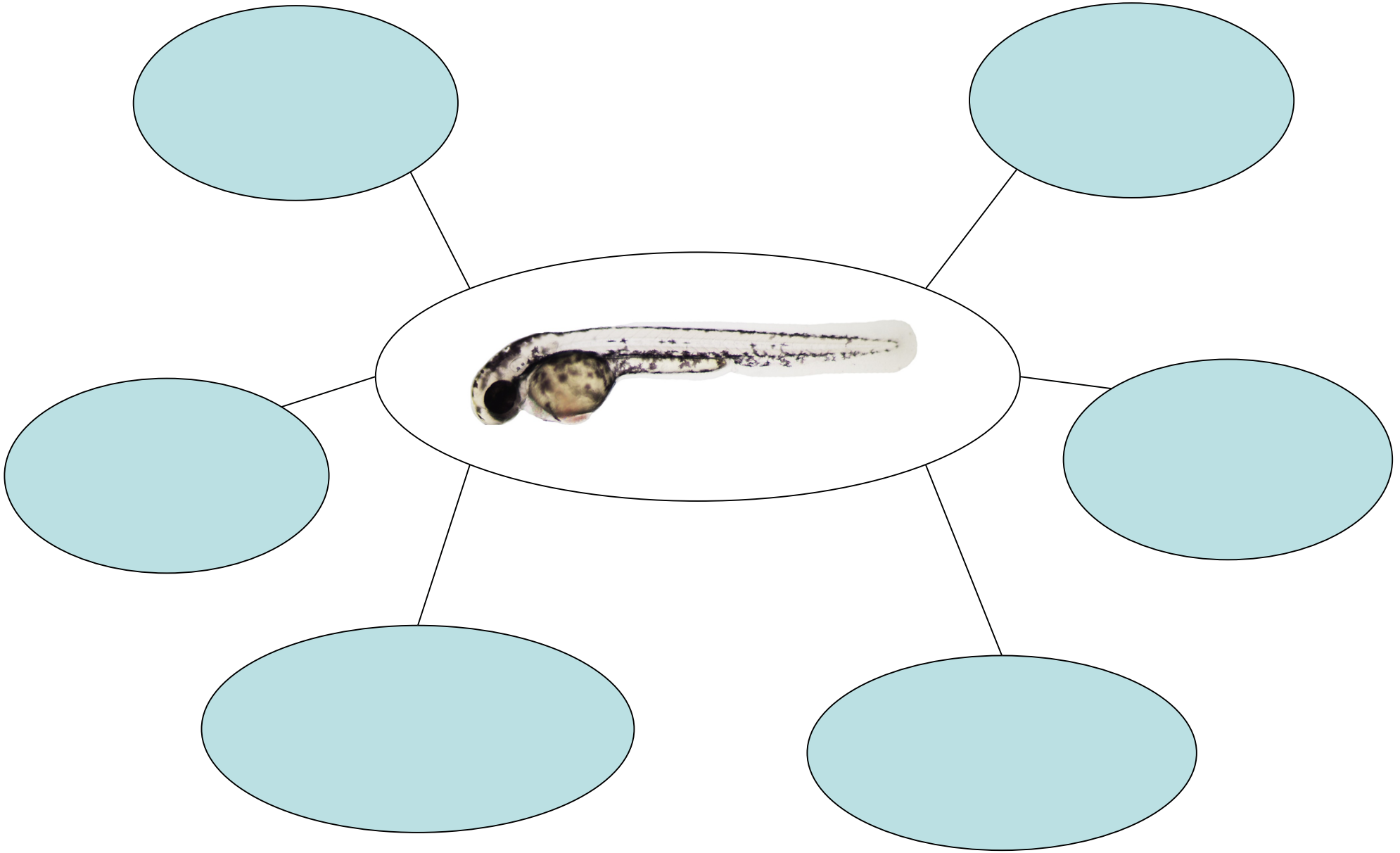


What was the goal of this study?



To identify compounds which increased beta-cell proliferation

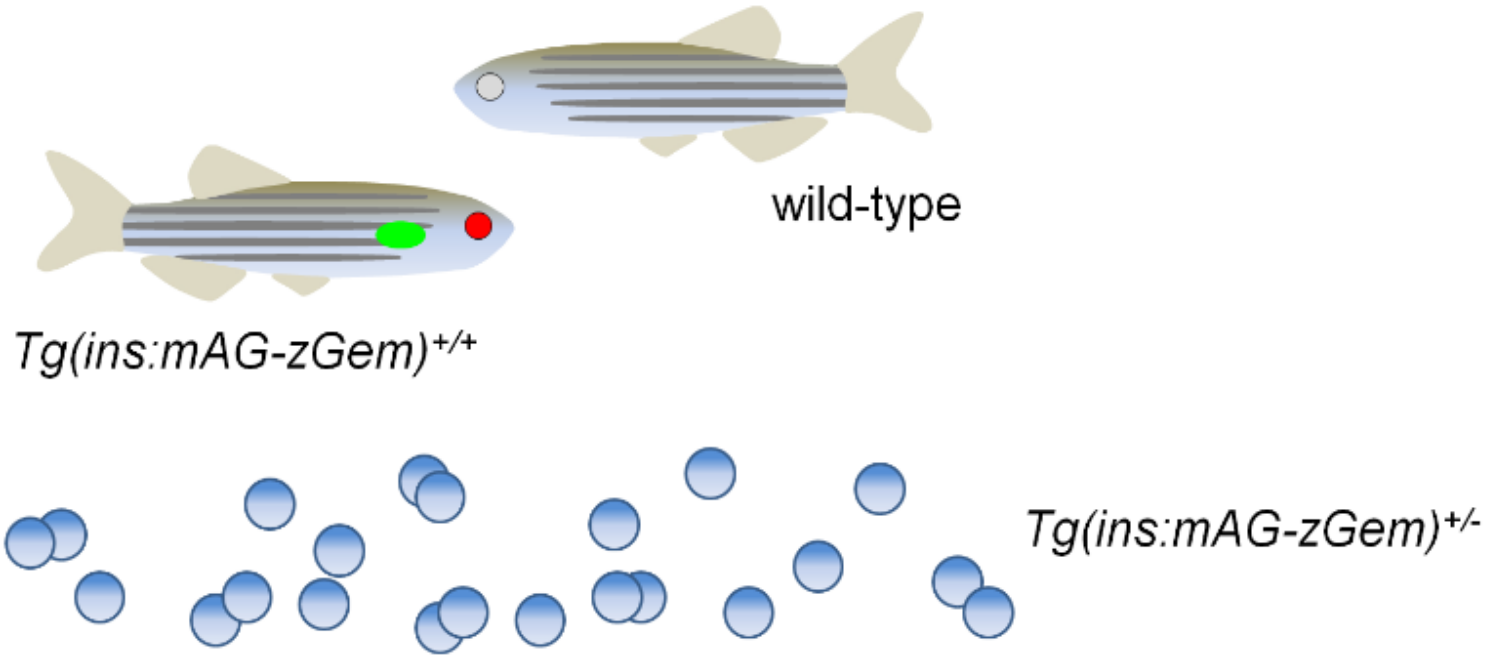
What are some benefits of utilizing Zebrafish larvae?



What are some benefits of utilizing Zebrafish larvae?

- Transparency
- Many offspring
- Cheap compared to mice
- Fast rate of development
- Same major organs and tissues as Humans
- Genetic structure similar to humans

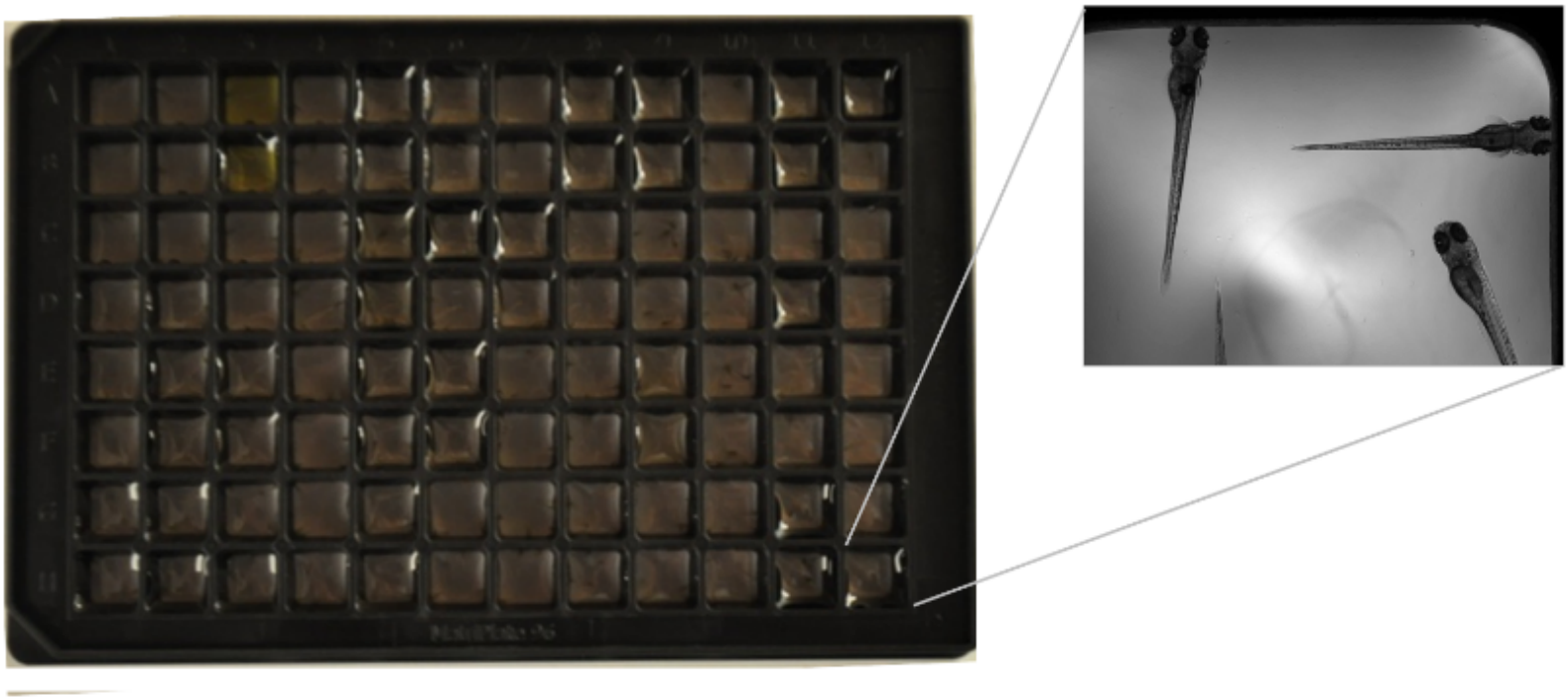
How were zebrafish larvae lines with beta-cell fluorescence created?



Homozygous transgenic fish are bred to wild-type fish to produce hundreds of hemizygous transgenic embryos.

Developing until 72 hpf

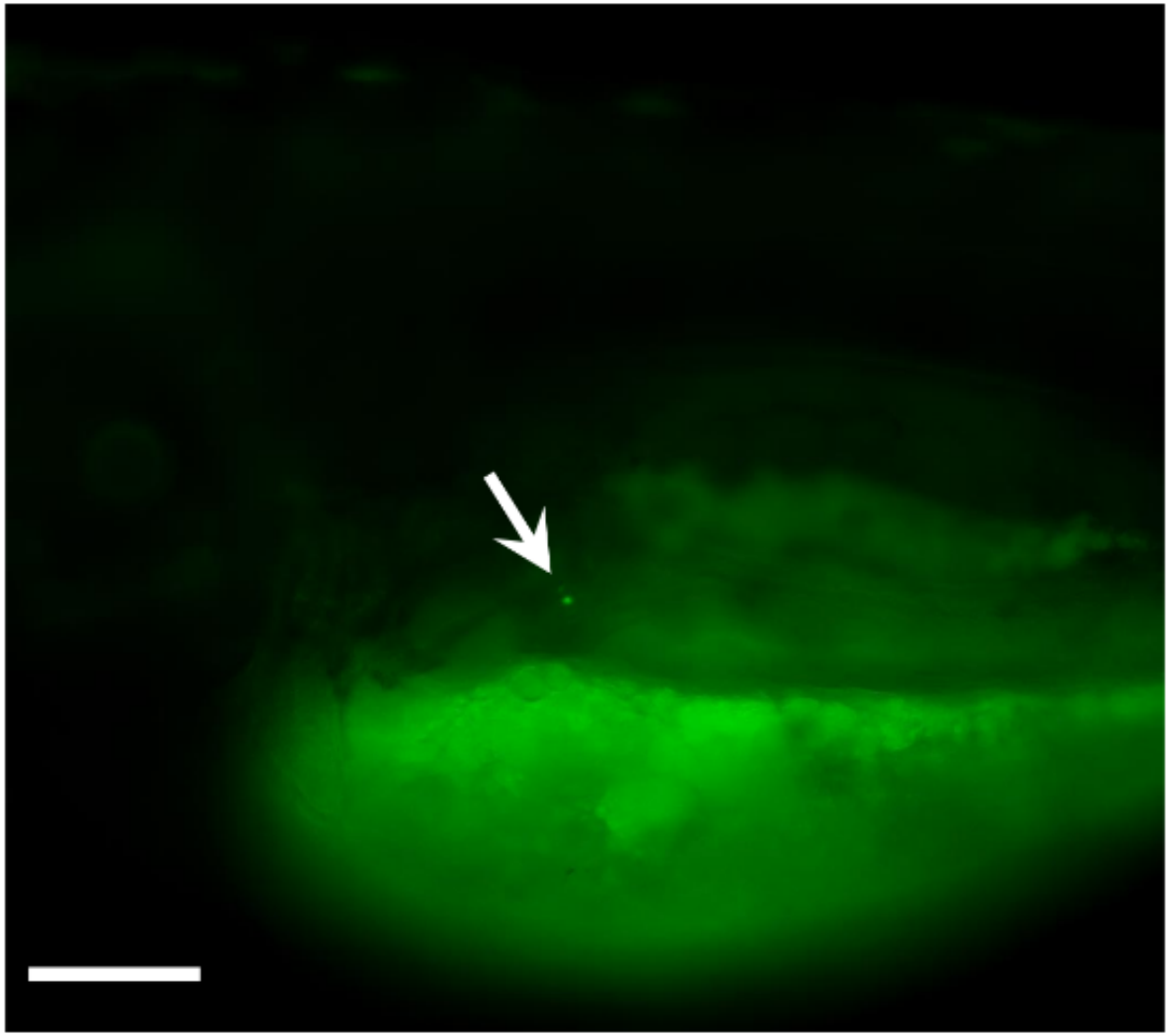
How did they screen for compounds that promoted beta-cell proliferation?



Tg(ins:mAG-zGem)$^{+/-}$ larvae are arrayed in 96-well plates (4 fish/well), each well containing a different chemical compound at 10 μM.

Chemical treatment for 24 hours

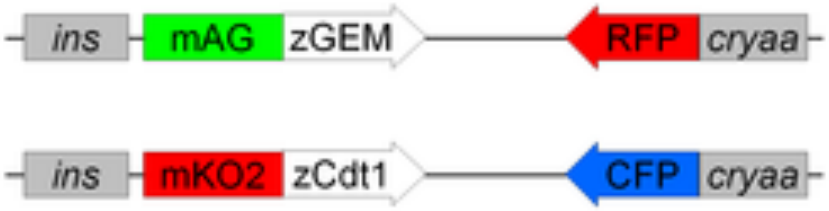
What did the proliferating beta-cells look like?



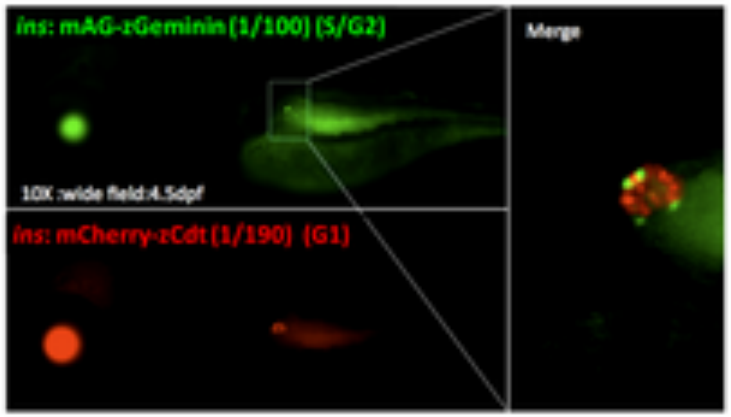
The proliferating beta-cells (green cell marked by an arrow) are counted under an inverted microscope

How did they differentiate proliferating and non proliferating beta-cells?

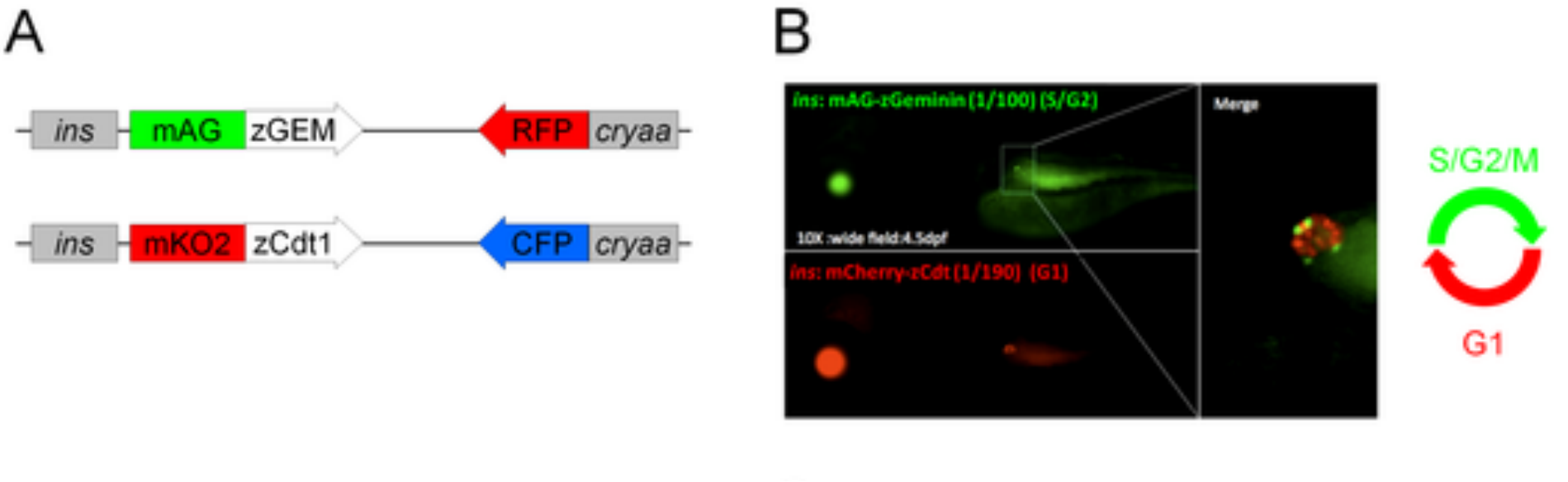
A



B



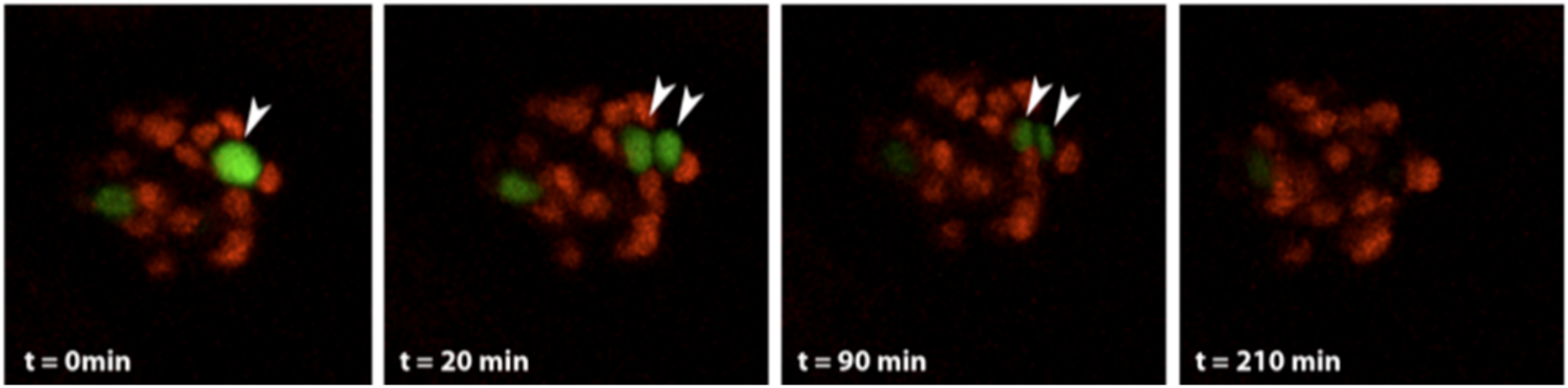
How did they differentiate proliferating and non proliferating beta-cells?



Proliferating beta-cells were labeled with green fluorescence while non-proliferating were labeled in red fluorescence

Did dividing beta-cells maintain expression in their daughter cells after mitosis?

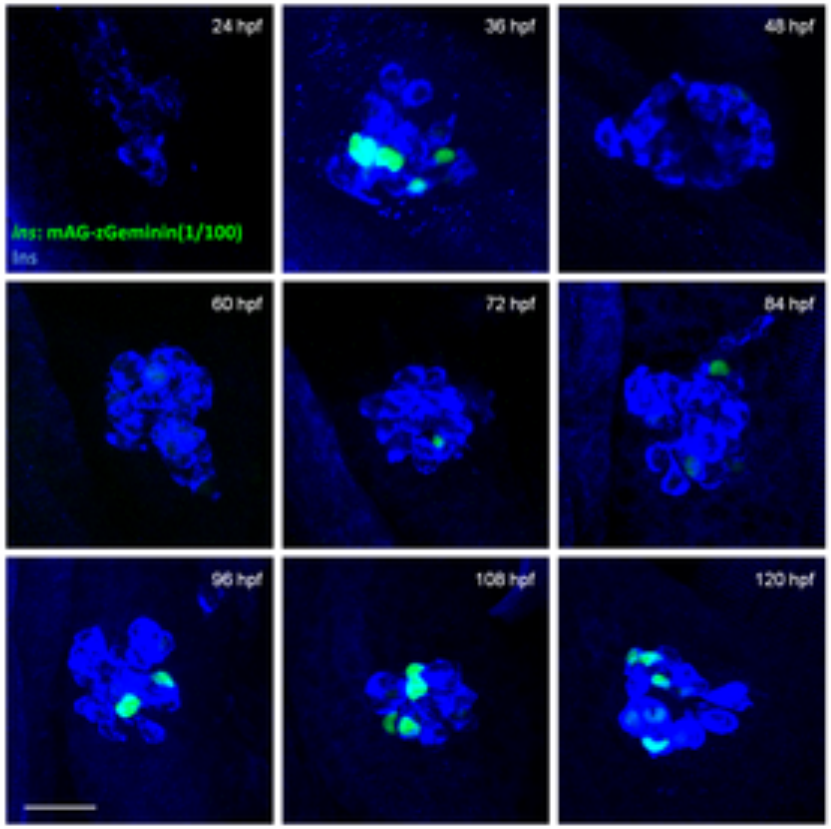
C



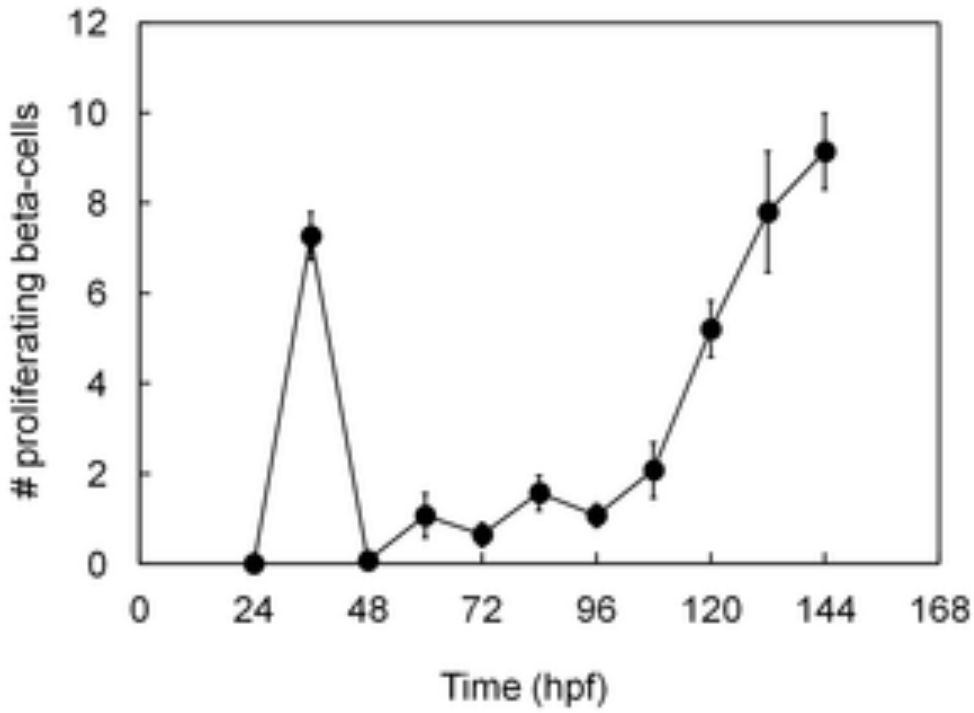
Dividing beta-cells were able to maintain their expression for over an hour past mitosis

At what points during zebrafish larval development are there natural increases in beta-cell proliferation?

E

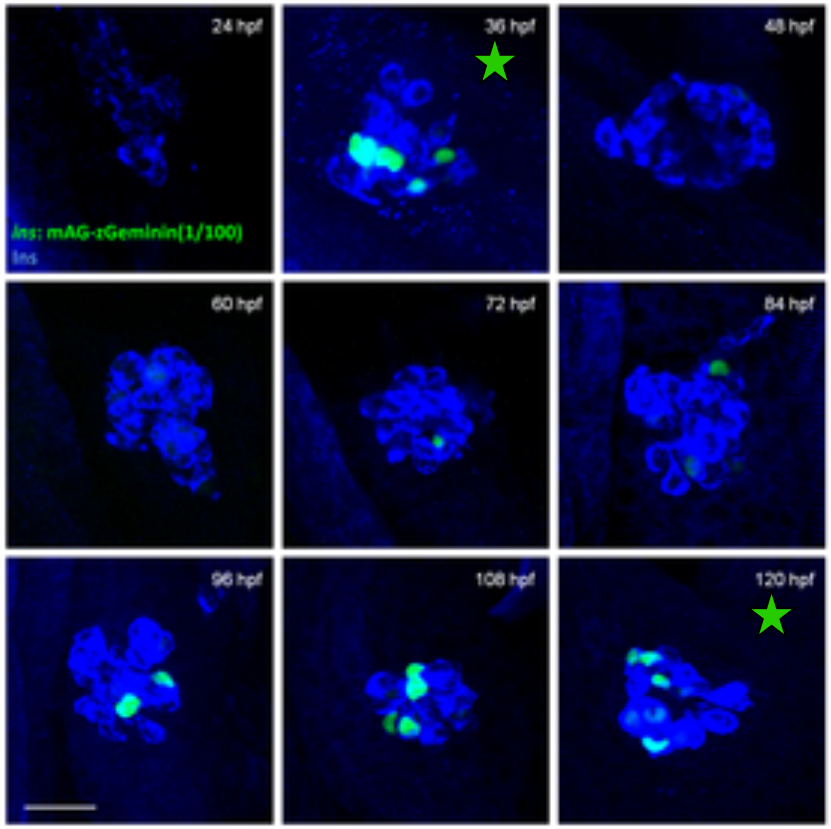


F

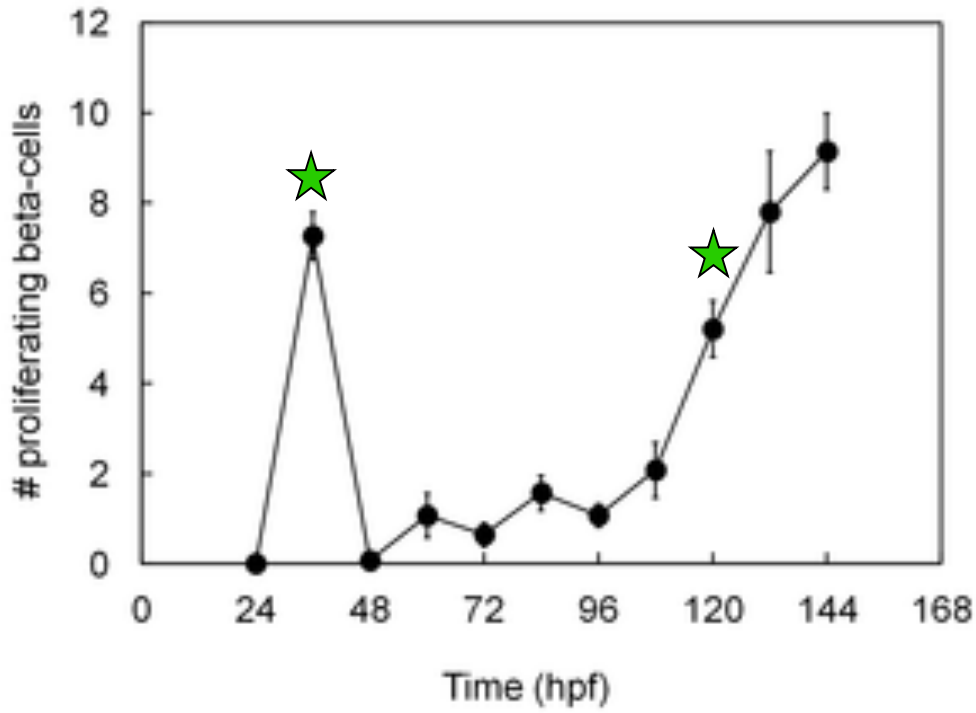


At what points during zebrafish larval development are there natural increases in beta-cell proliferation?

E



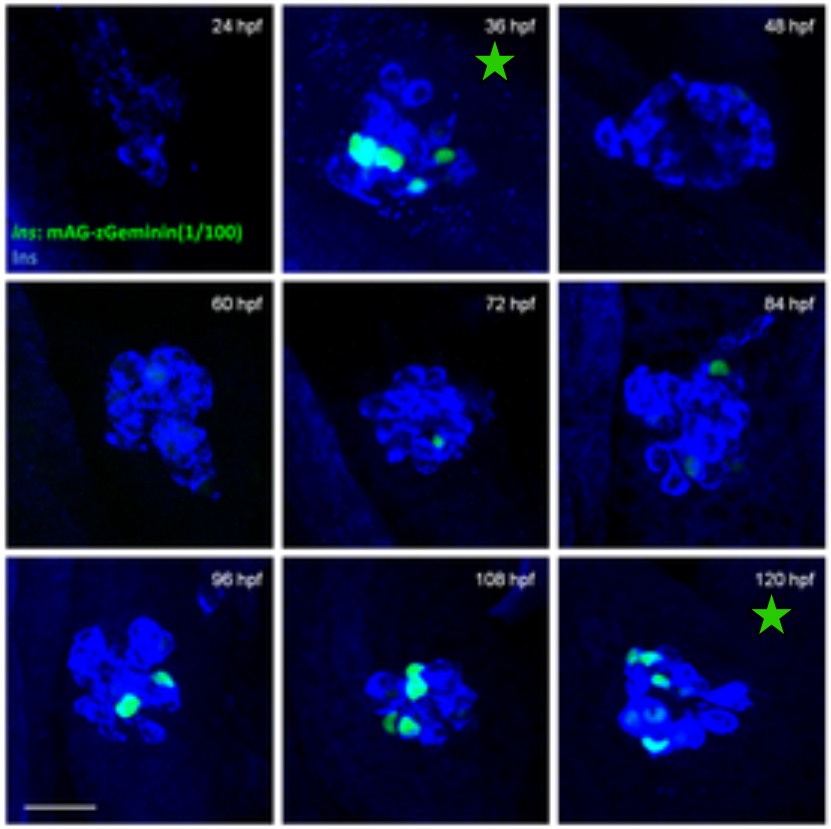
F



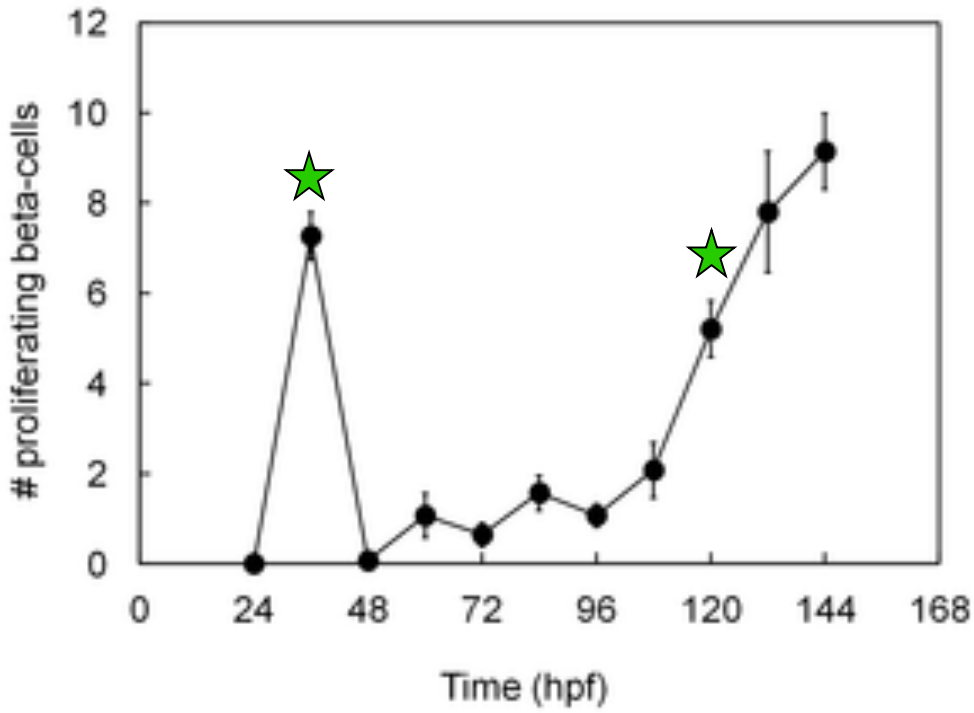
Peaks in beta-cell proliferation were observed at 36 hpf and 120 hpf

What did these natural increases in beta-cell proliferation correlate with?

E



F



Peaks in beta-cell proliferation coincided with periods when glucose levels were increased

Which compound served as a control?

Classification	Chemical Name	# mAG$^+$ cells	Pharmacological Action	Dose (μM)	Library
Control	DMSO	0.5±0.06			
Retinoic acid	Retinoic acid, all trans	5.0±0.7	RAR agonist	10	NRL
	4-Hydroxyretinoic acid	3.7±0.9	Retinoid metabolite	10	NRL
	9-cis Retinoic acid	2.5±0.5	RXR agonist	10	NRL
	Isotretinoin (13-cis-Retinoic Acid)	2.5±0.5	RAR agonist	10	NIH II
Serotonin	Trazodone	1.3±0.3	Serotonin uptake inhibitor	10	NIH I
	Lofepramine	1.0±0.3	Serotonin and noradrenalin re-uptake inhibitor	10	NIH I
	Phenelzine	1.1±0.3	Monoamine oxidase inhibitor	10	NIH II
Glucocorticoids	Fluticasone	2.7±0.6	Glucocorticoid receptor ligands	10	NIH I
	Hydrocortisone acetate	2.8±0.7		10	NIH II
	Prednisolone acetate	2.9±0.7		10	NIH II
	Clobetasol propionate	2.7±0.5		10	NIH II
	Triamcinolone acetonide	2.9±0.5		10	NIH II
	Westcort	2.6±0.4		10	NIH II
	Budesonide	3.2±0.4		10	NIH II
	Methylprednisolone acetate	2.0±0.4		10	NIH II
	Fluocinolone acetonide 21-acetate	2.3±0.4		10	NIH II
	Dexamethasone	1.4±0.3		10	NIH II
	Cortisone acetate	1.2±0.3		10	NIH II
	Amcinonide	2.1±0.4		10	NIH II
	Fluocinolone acetonide	1.7±0.3		10	NIH II

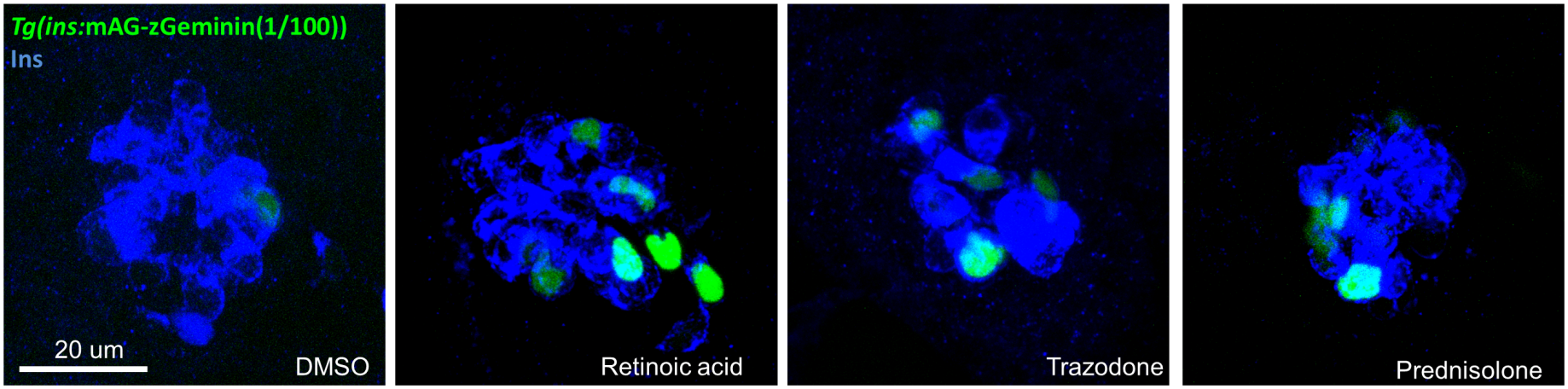
What criteria determined if a compound significantly increased beta-cell proliferation?

Classification	Chemical Name	# mAG$^+$ cells	Pharmacological Action	Dose (μM)	Library
Control	DMSO	0.5±0.06			
Retinoic acid	Retinoic acid, all trans	5.0±0.7	RAR agonist	10	NRL
	4-Hydroxyretinoic acid	3.7±0.9	Retinoid metabolite	10	NRL
	9-cis Retinoic acid	2.5±0.5	RXR agonist	10	NRL
	Isotretinoin (13-cis-Retinoic Acid)	2.5±0.5	RAR agonist	10	NIH II
Serotonin	Trazodone	1.3±0.3	Serotonin uptake inhibitor	10	NIH I
	Lofepramine	1.0±0.3	Serotonin and noradrenalin re-uptake inhibitor	10	NIH I
	Phenelzine	1.1±0.3	Monoamine oxidase inhibitor	10	NIH II
Glucocorticoids	Fluticasone	2.7±0.6	Glucocorticoid receptor ligands	10	NIH I
	Hydrocortisone acetate	2.8±0.7		10	NIH II
	Prednisolone acetate	2.9±0.7	Glucocorticoid receptor ligands	10	NIH II
	Clobetasol propionate	2.7±0.5		10	NIH II
	Triamcinolone acetonide	2.9±0.5		10	NIH II
	Westcort	2.6±0.4		10	NIH II
	Budesonide	3.2±0.4		10	NIH II
	Methylprednisolone acetate	2.0±0.4		10	NIH II
	Fluocinolone acetonide 21-acetate	2.3±0.4		10	NIH II
	Dexamethasone	1.4±0.3		10	NIH II
	Cortisone acetate	1.2±0.3		10	NIH II
	Amcinonide	2.1±0.4		10	NIH II
	Fluocinolone acetonide	1.7±0.3		10	NIH II

Compounds had to have a 2x increase in beta-cell proliferation compared to DMSO control

Does the concentration of the compound effect the number of observed proliferating beta-cells?

A



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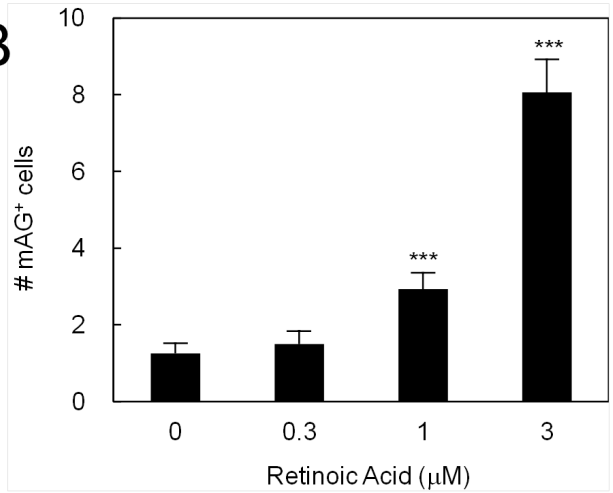
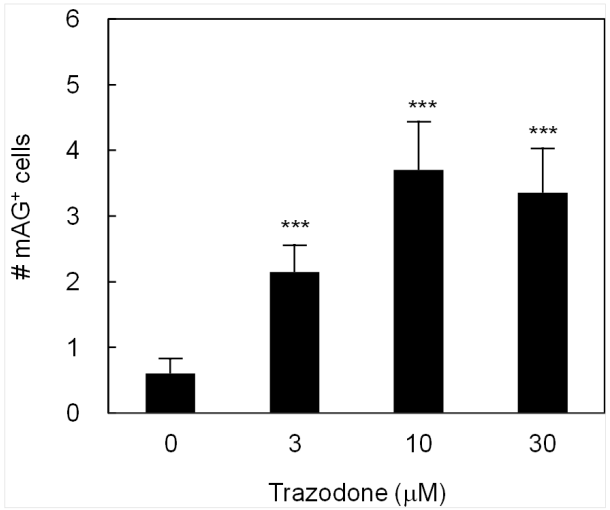
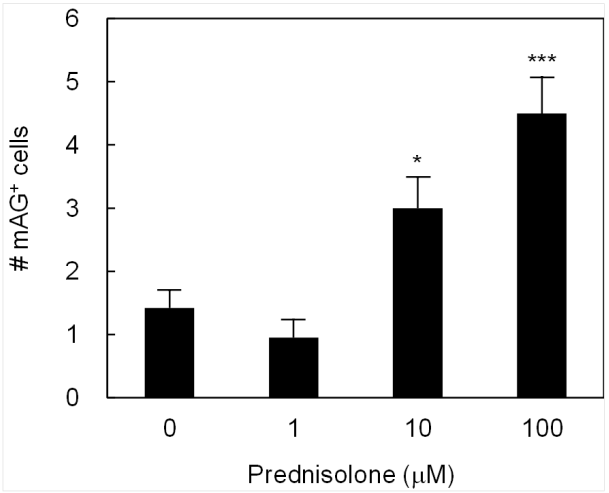
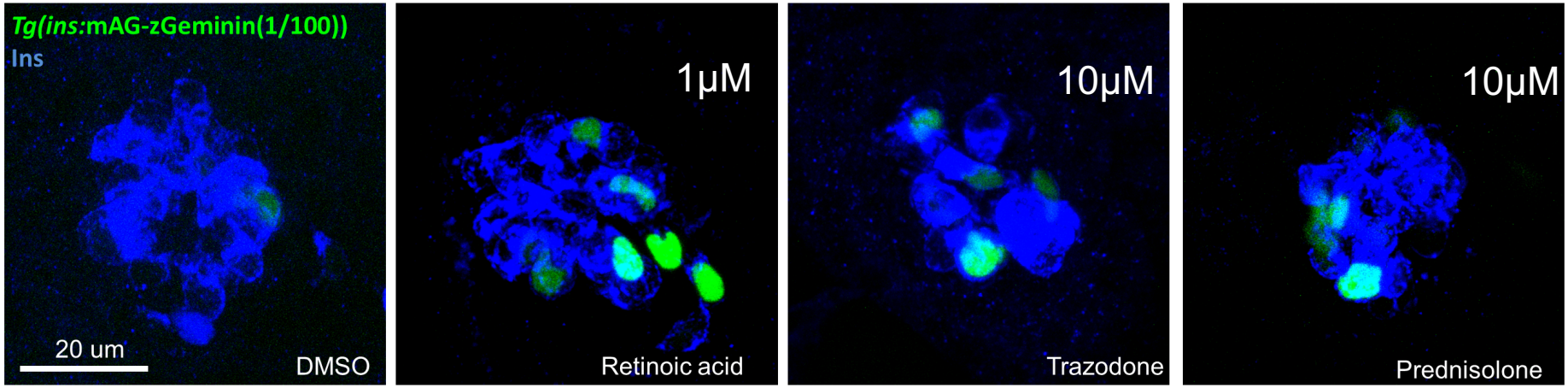




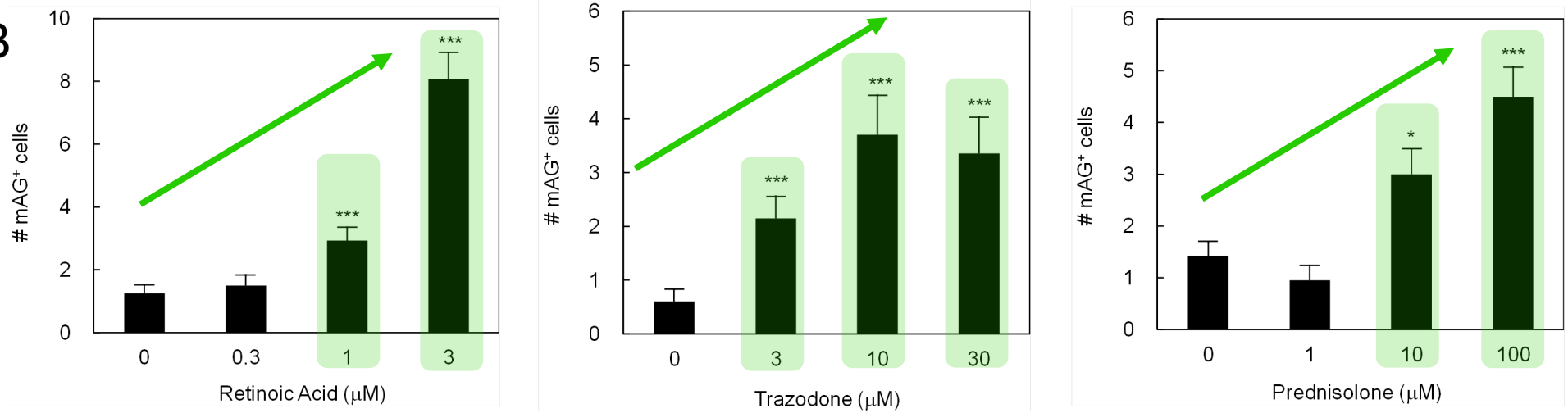


Does the concentration of the compound effect the number of observed proliferating beta-cells?

A

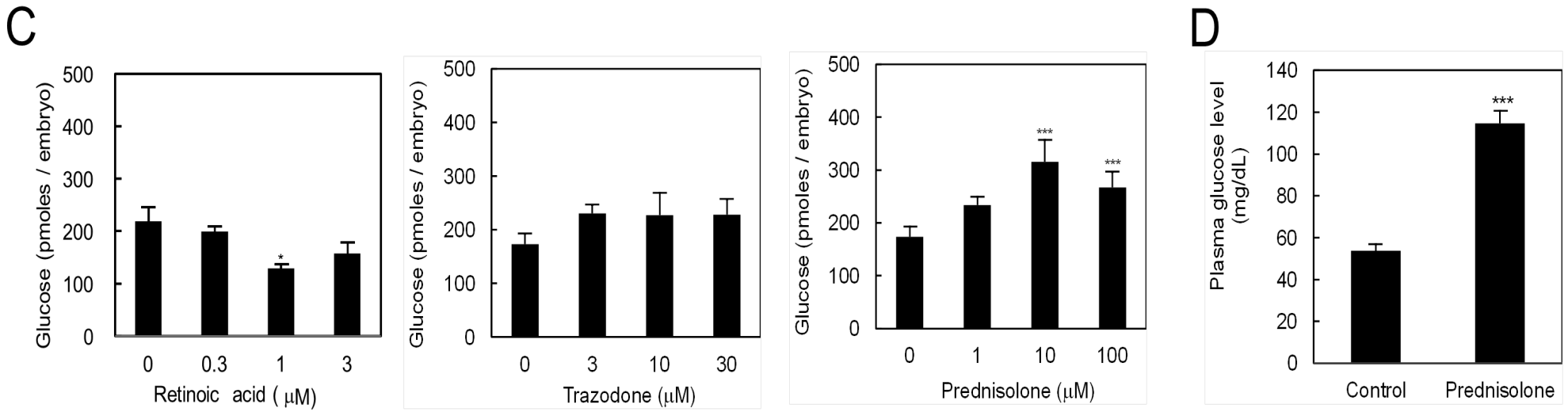


B



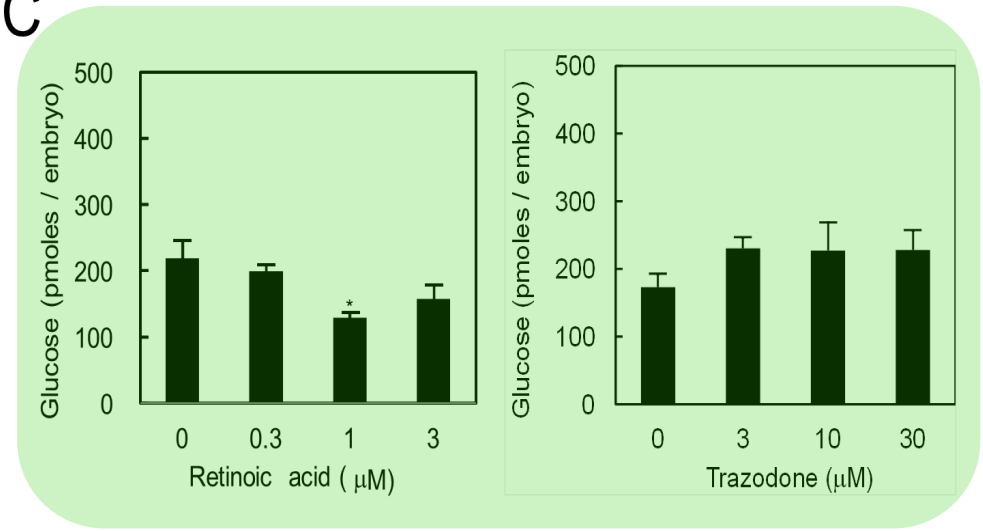
The number of proliferating beta-cells significantly increased with increasing concentrations

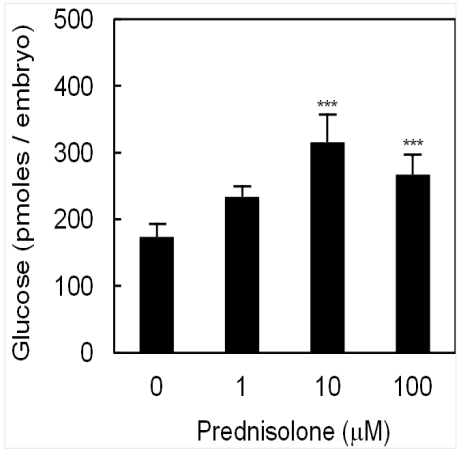
Did the compounds promote beta-cell proliferation without causing hyperglycemia?



Did the compounds promote beta-cell proliferation without causing hyperglycemia?

C





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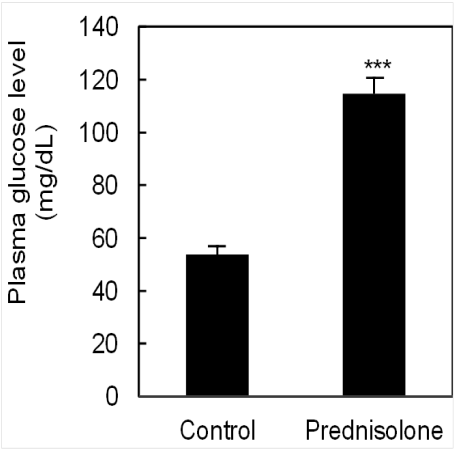
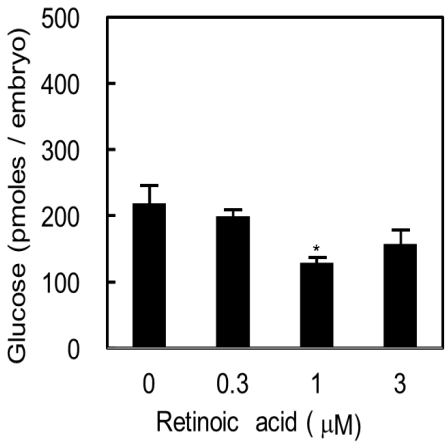
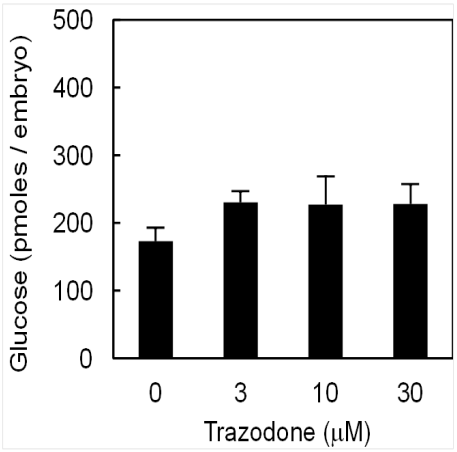


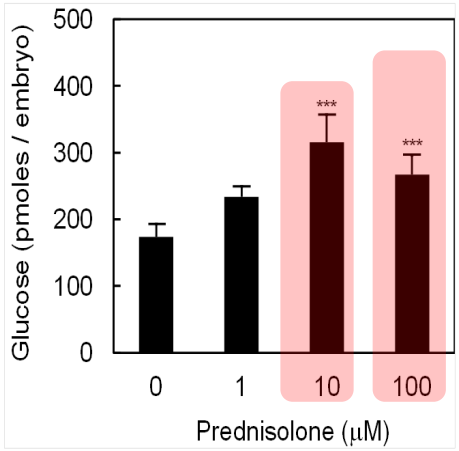
Retinoic acid and Trazodone promoted beta cell proliferation without causing hyperglycemia

Did the compounds promote beta-cell proliferation without causing hyperglycemia?

C







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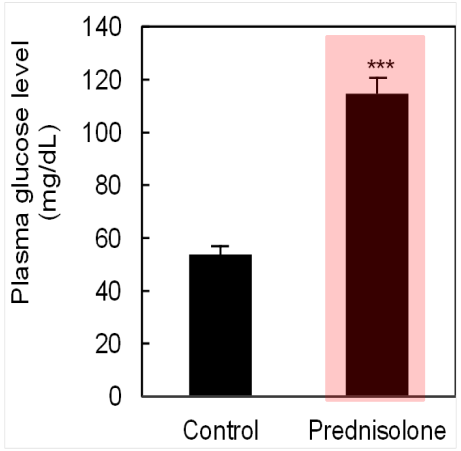
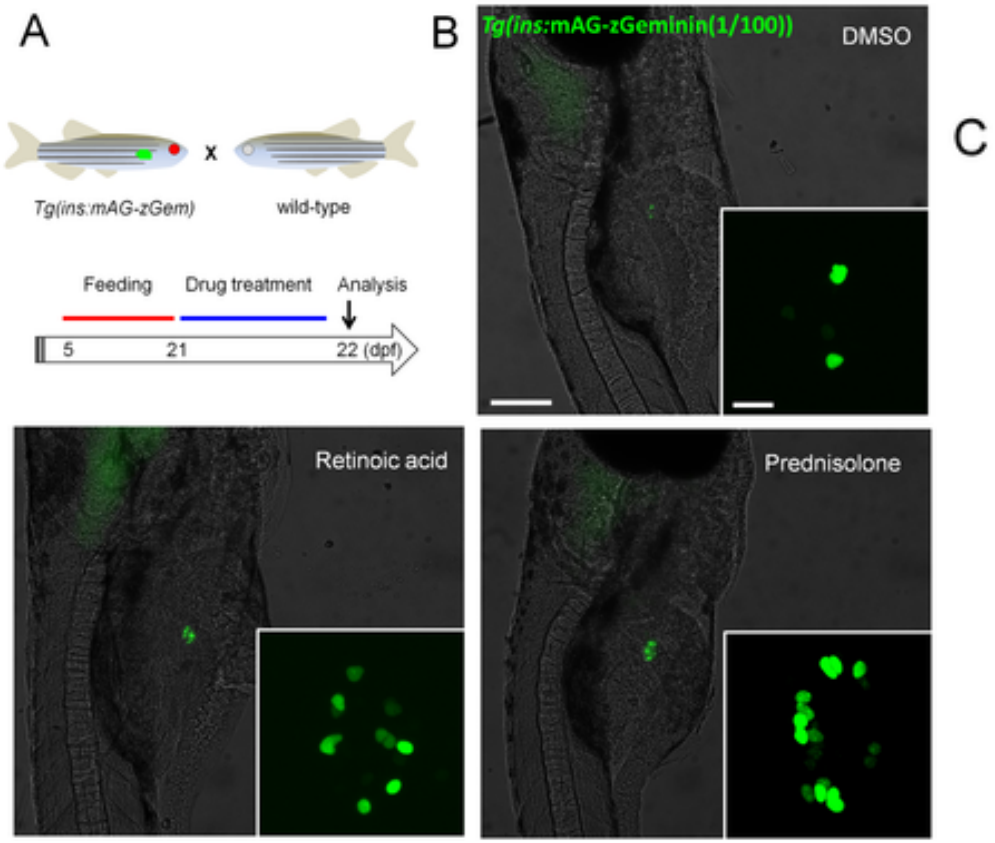
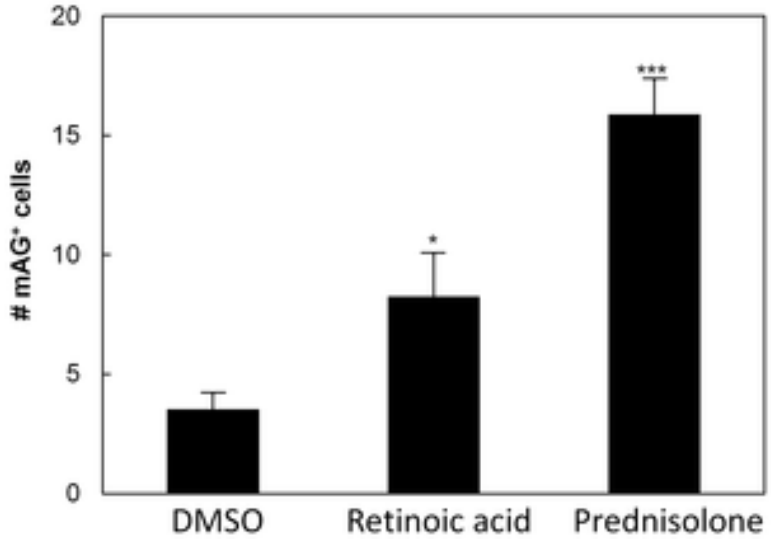


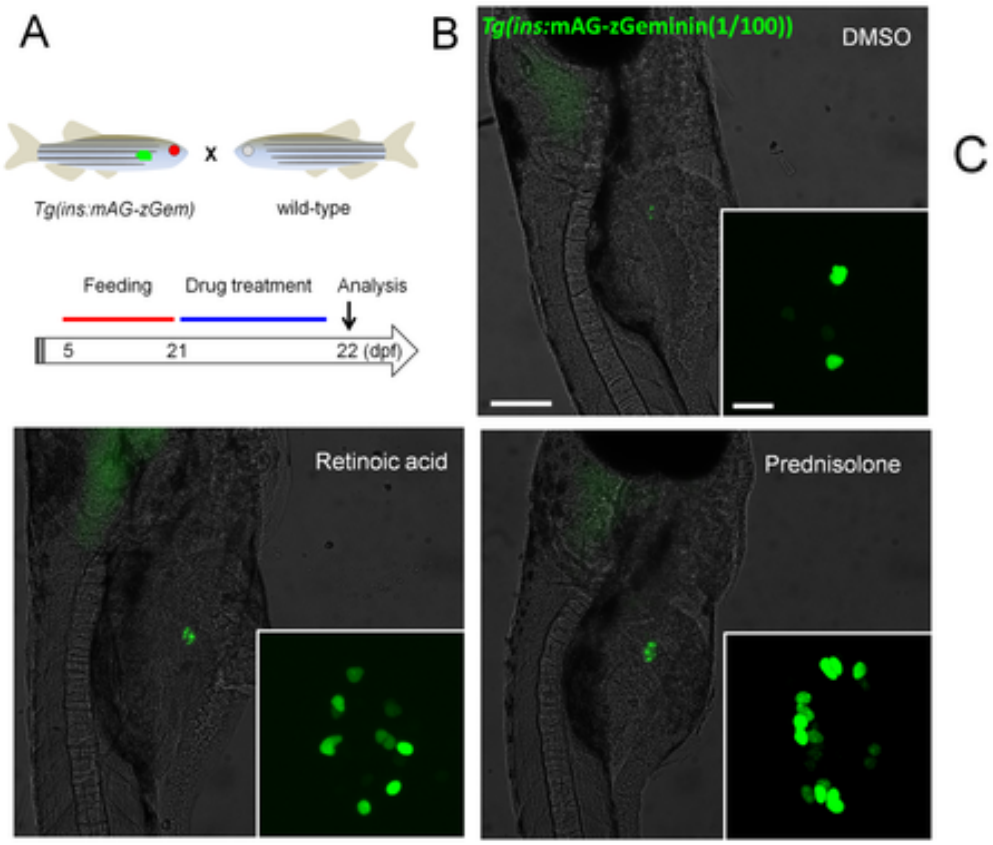
Prednisolone promoted beta-cell proliferation, but caused hyperglycemia

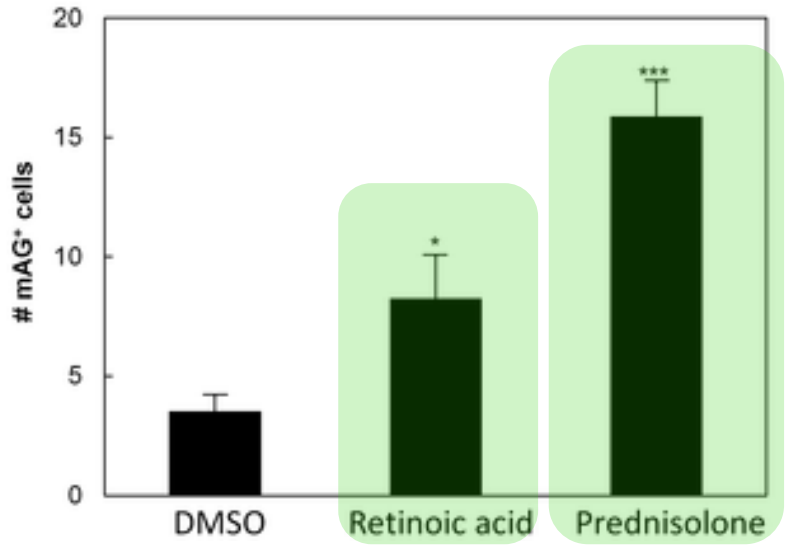
How do retinoic acid and prednisolone effect pancreatic beta-cell proliferation during feeding metabolism?





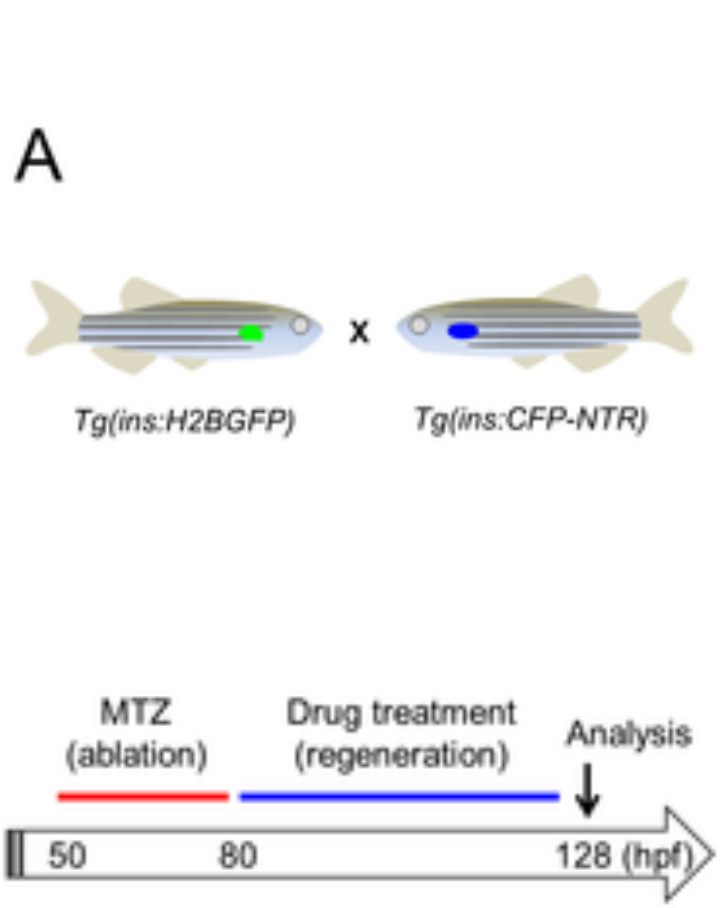
How do retinoic acid and prednisolone effect pancreatic beta-cell proliferation during feeding metabolism?

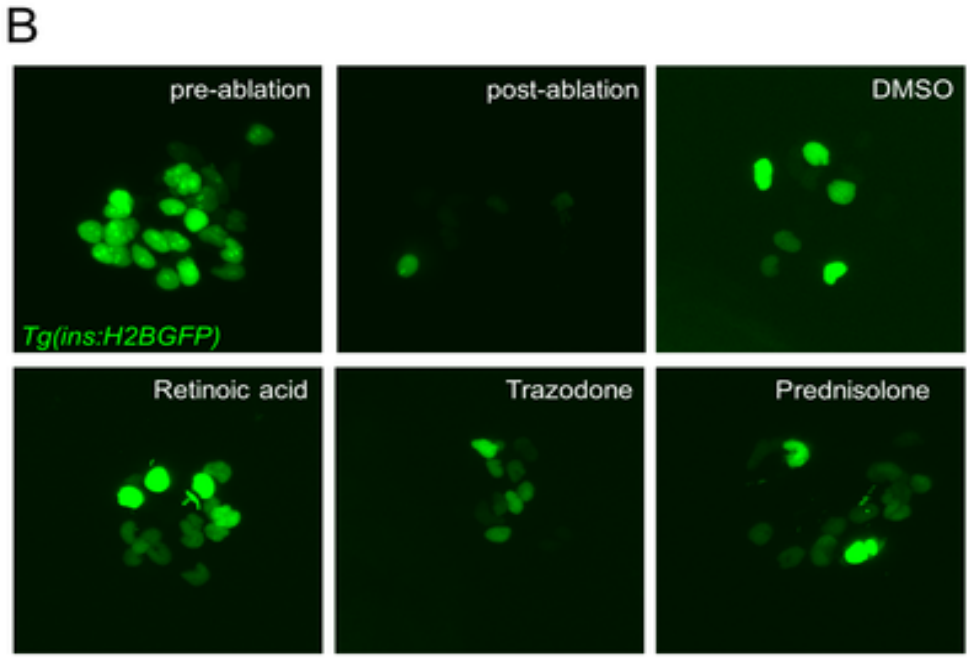


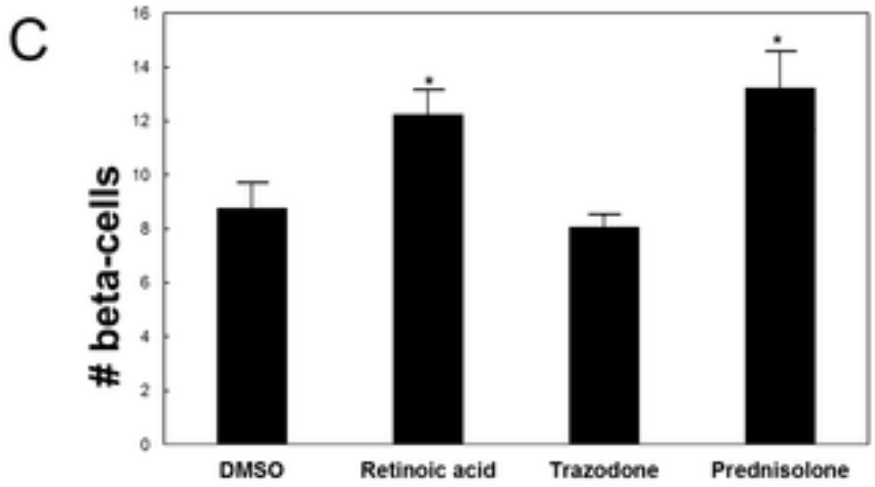


Retinoic acid and prednisolone significantly increase beta-cell proliferation during feeding metabolism

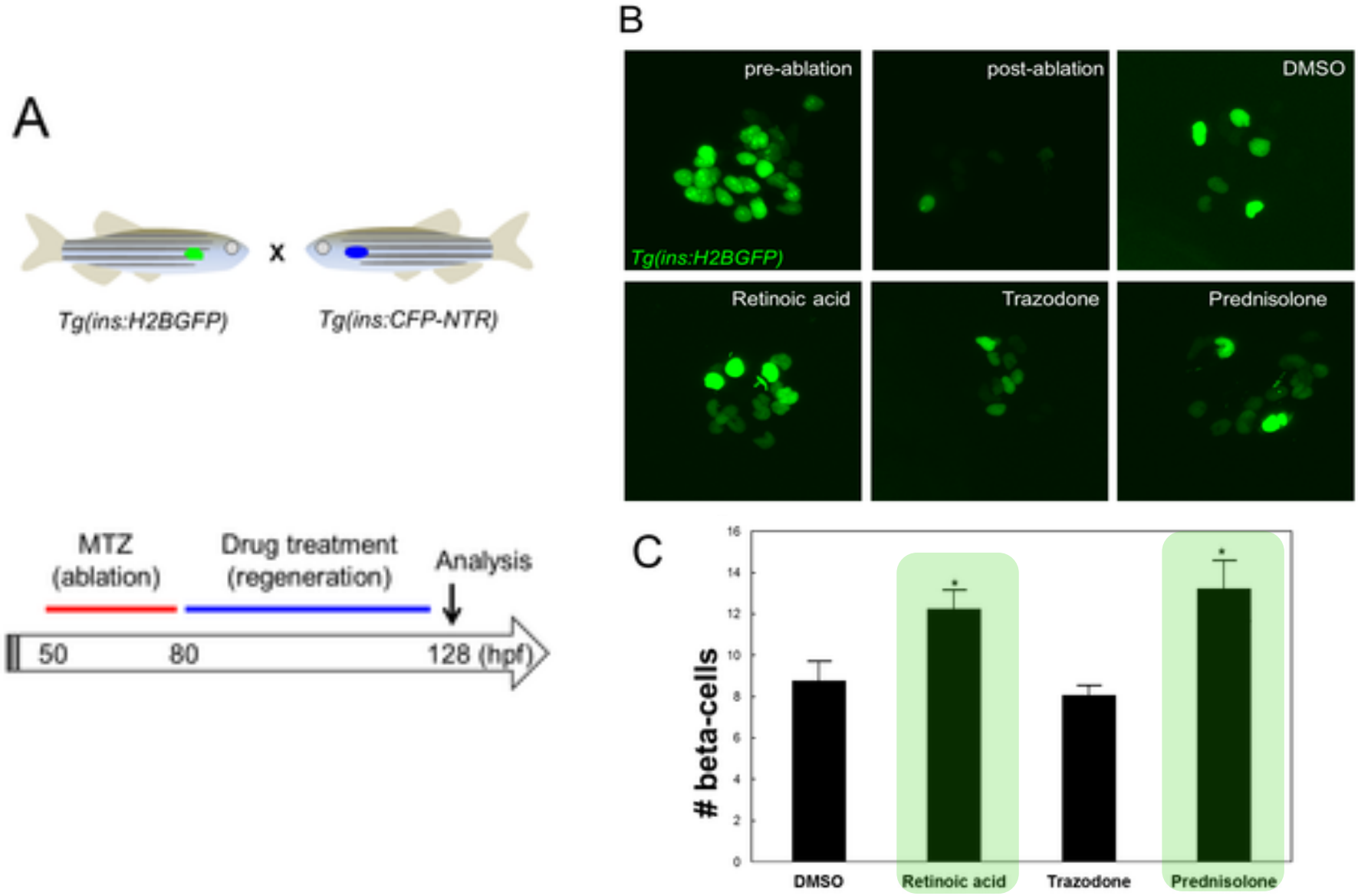
Could the compounds also promote beta-cell regeneration?





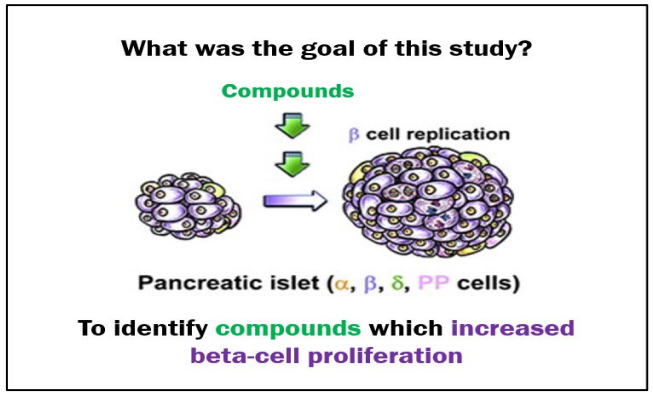


Could the compounds also promote beta-cell regeneration?

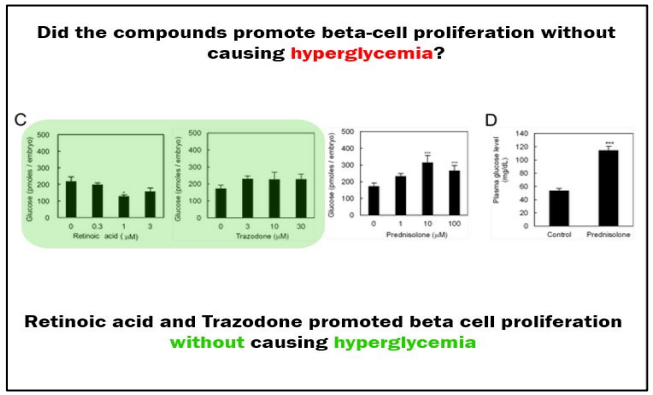


Retinoic acid and Prednisolone significantly promoted beta-cell regeneration

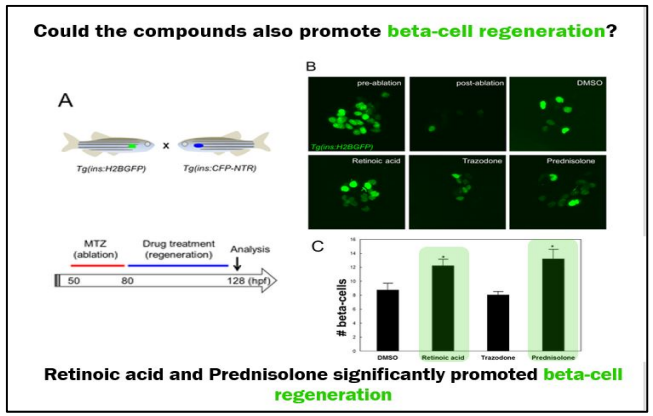
Summary



Compounds which increased beta-cell proliferation were identified

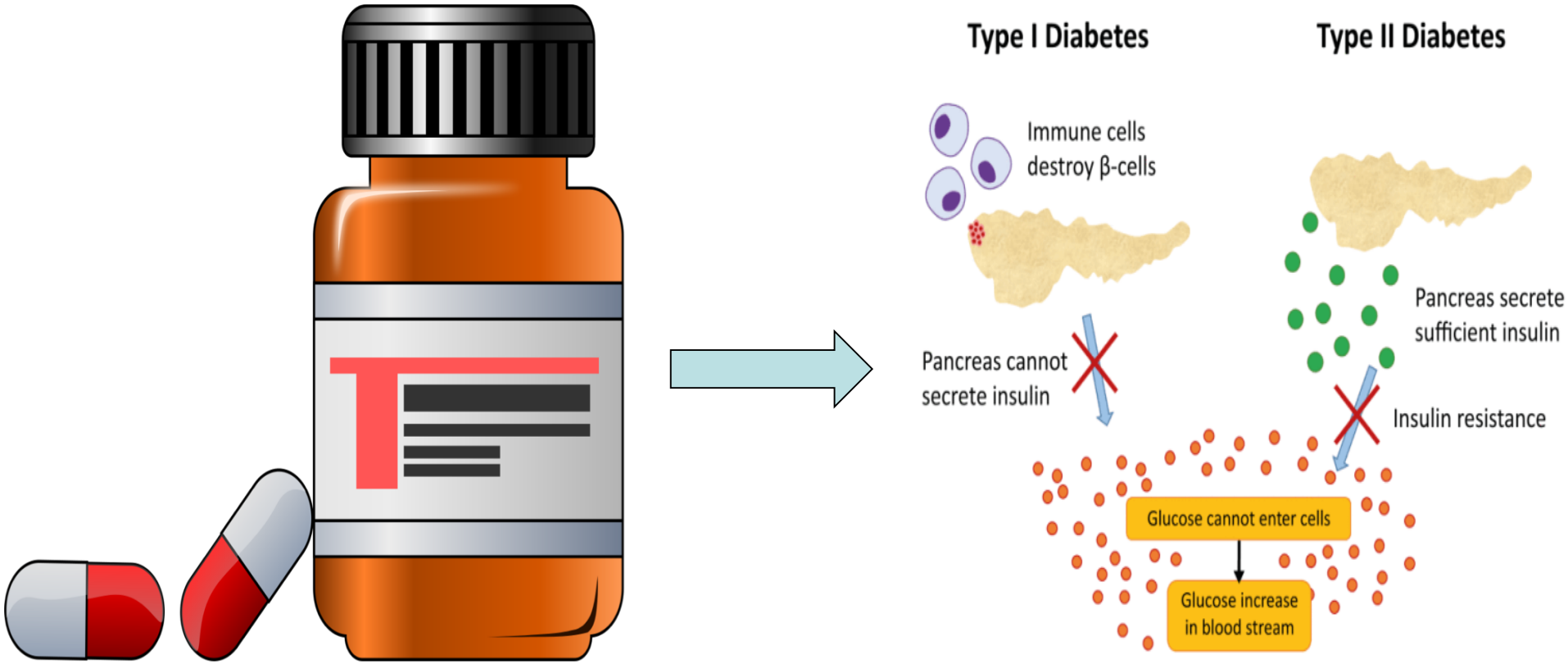


Retinoic acid and Trazadone promoted beta-cell proliferation without hyperglycemia

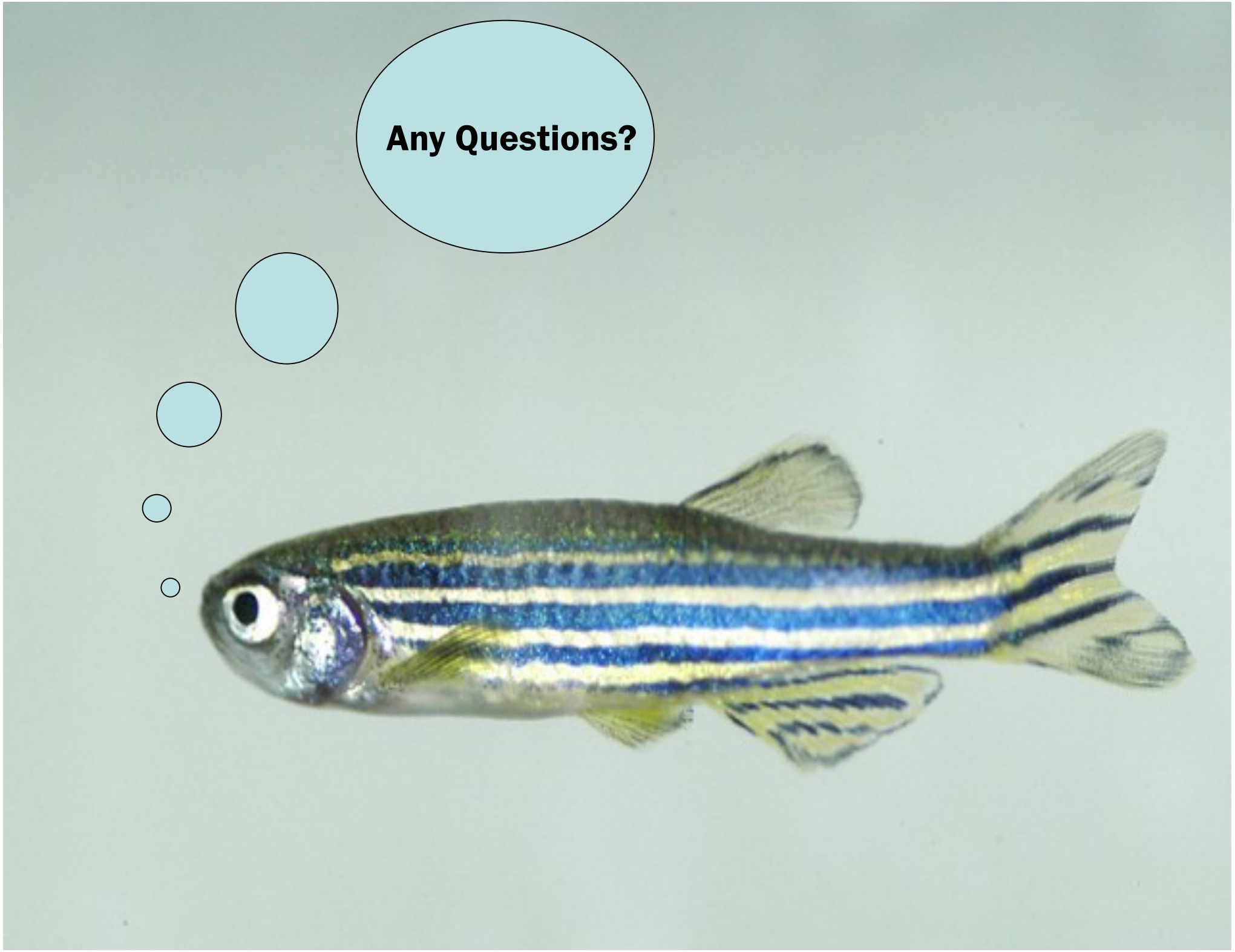


Retinoic acid and Prednisolone even promoted beta-cell regeneration

What is the importance of this study?



New therapeutic drugs to promote beta-cell proliferation and regeneration in diabetics


Any Questions?

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