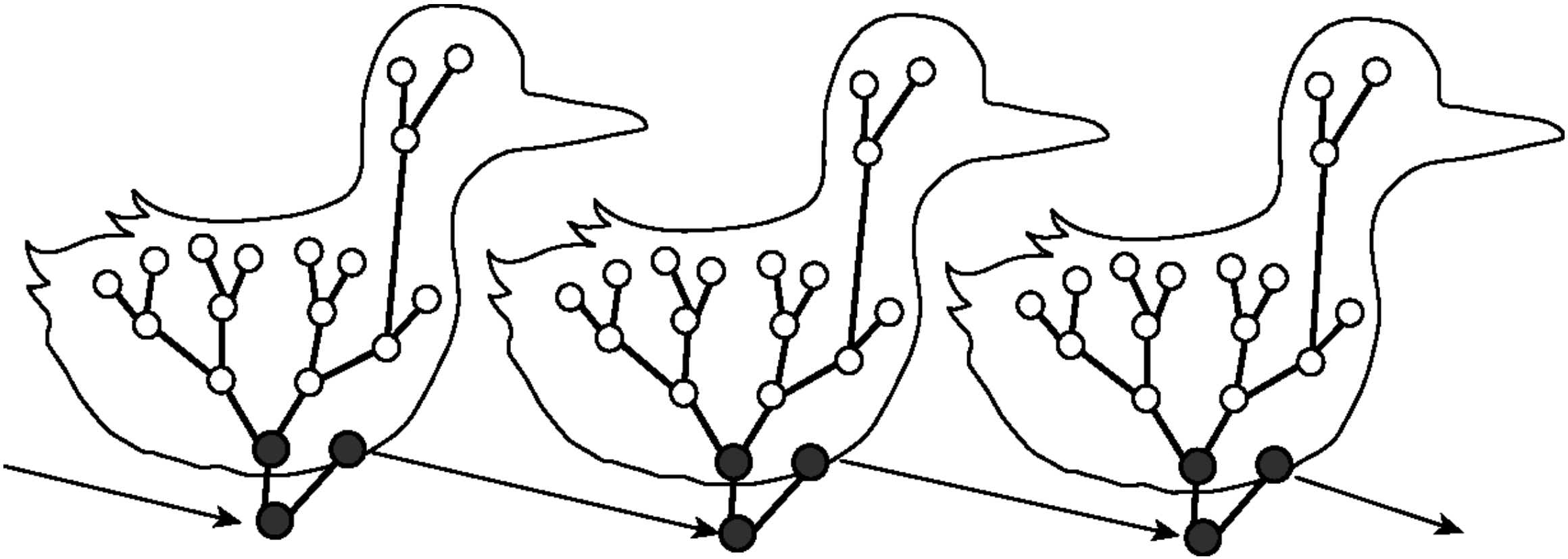
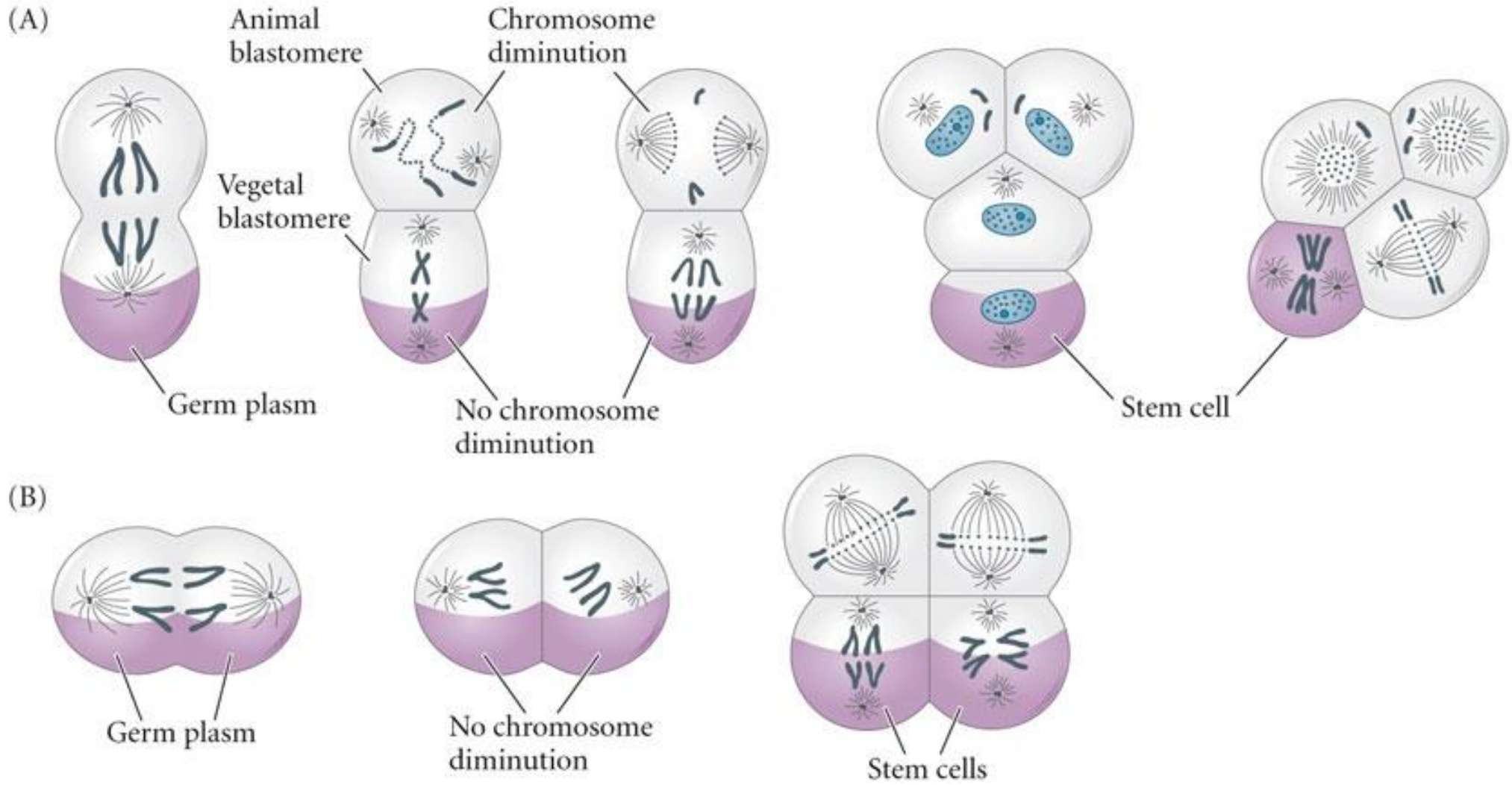
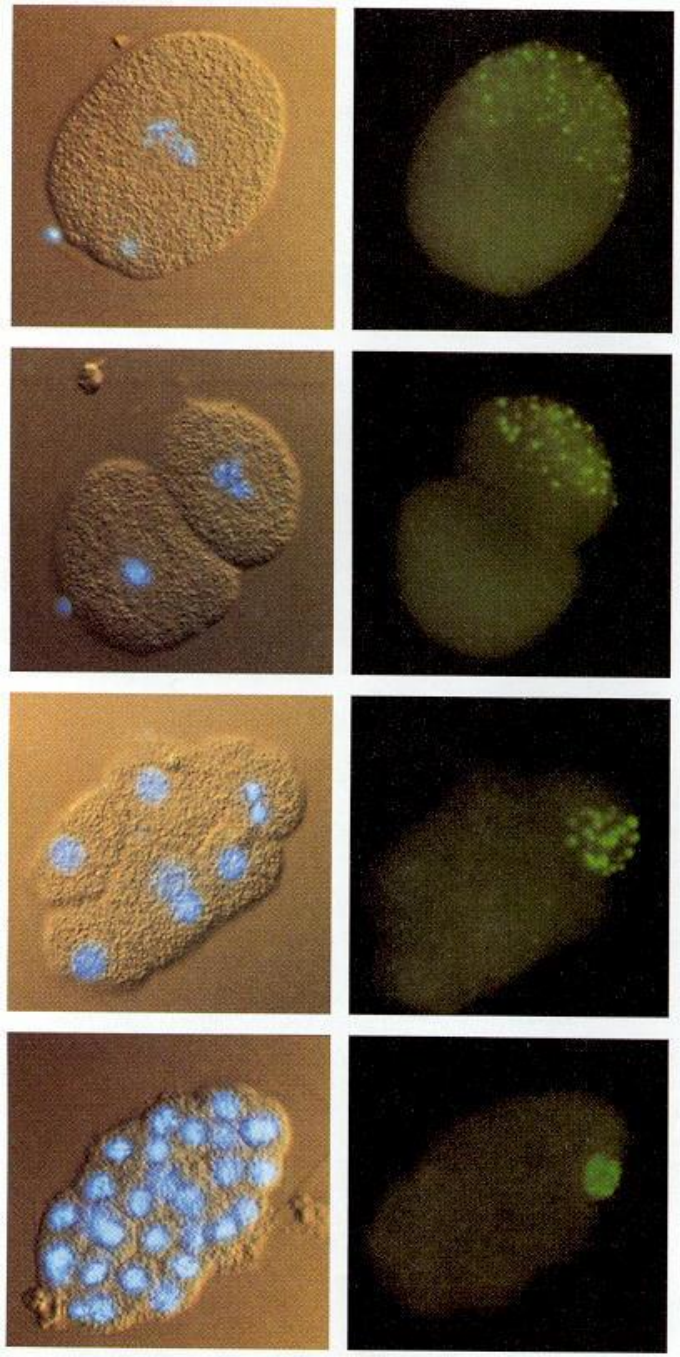
Células madre y línea germinal



Uriel Koziol
ukoziol@fcien.edu.uy

Boveri: disminución cromosómica en *Parascaris*





Gránulos P (P-granules) en la especificación de la línea germinal de *Caenorhabditis elegans*

Complejos ribonucleoproteicos con determinantes para la línea germinal

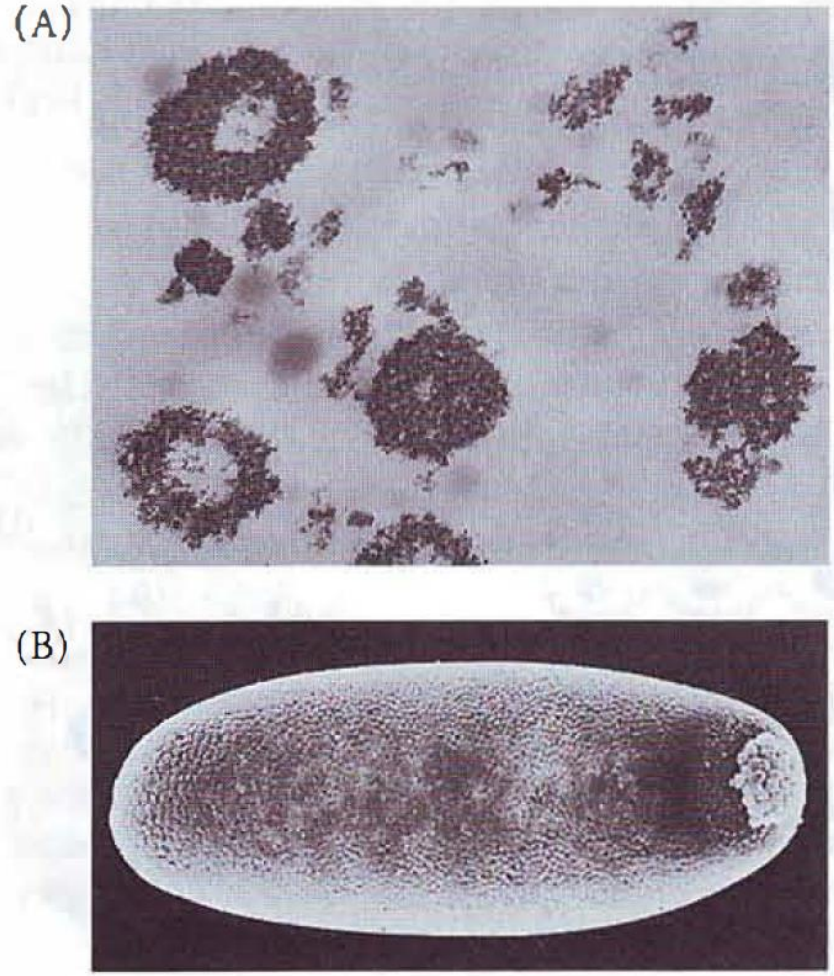


FIGURE 16.3 Pole plasm of *Drosophila*. (A) Electron micrograph of polar granules from particulate fraction of *Drosophila* pole cells. (B) Scanning electron micrograph of a *Drosophila* embryo just prior to completion of cleavage. The pole cells can be seen at the right of the photograph. (Photographs courtesy of A. P. Mahowald.)

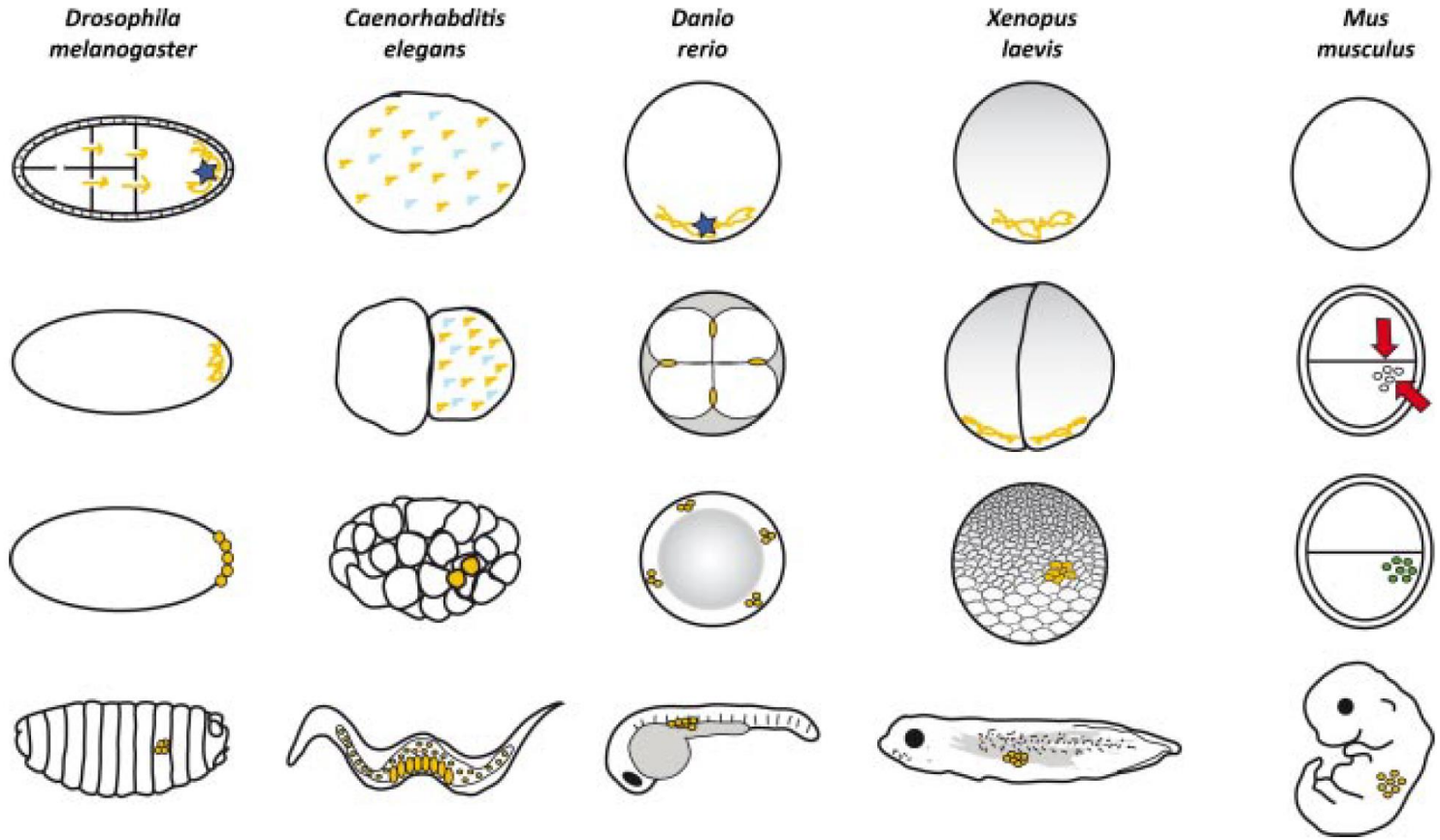


Figure 1. Localization of germ line specification molecules throughout animal development. Developmental progression in time goes from top to bottom. Germ cell specification and the localization of molecules discussed in this review are schematized for the five genetic laboratory organisms that have contributed the most to our current understanding of the molecular mechanisms underlying germ cell specification. In all organisms except for mice (*Mus musculus*), germ cell-specific gene products (yellow), including mRNAs and/or proteins of *vasa*, *nanos*, *pumilio*, *piwi*, and *tudor*, are localized to the cytoplasm of germ cells either late in oogenesis or early during embryogenesis. The fruit fly (*Drosophila melanogaster*) protein Oskar and the zebrafish (*Danio rerio*) protein Bucky ball (dark blue star) can autonomously assemble many of these germ plasm components in oocytes and early embryos. The nematode (*Caenorhabditis elegans*) protein PIE-1 (light blue) plays an important role in regulating germ line gene expression (yellow). In mice, somatic signals (red) trigger the expression of Blimp1 (green) in PGCs, followed by the expression of conserved germ line genes (yellow).

Table 1. The germline gene set: functions in ecdysozoan and chordate germlines

Gene	Key functional discoveries
vasa: deadbox helicase, translational regulator	Required for germline development in *Drosophila* (Schupbach and Wieschaus, 1986) Required for gametogenesis in *Drosophila*, *C. elegans* and mouse (Kuznicki et al., 2000; Styhler et al., 1998; Tanaka et al., 2000) Positive translational regulator of *gurken* and *oskar* in *Drosophila* (Breitwieser et al., 1996; Styhler et al., 1998; Tomancak et al., 1998) Interacts with the translational machinery (Carrera et al., 2000; Johnstone and Lasko, 2004)
nanos: zinc-finger protein, translational repressor	Required for germline development in *Drosophila, C. elegans* and mouse (Kobayashi et al., 1996; Subramaniam and Seydoux, 1999; Tsuda et al., 2003) Required for GSC maintenance in *Drosophila* and mouse (Bhat, 1999; Forbes and Lehmann, 1998; Sada et al., 2009; Wang and Lin, 2004) Required with *pumilio* to repress mitosis in *Drosophila* pole cells via the translational repression of *cyclin B* mRNA (Asaoka-Taguchi et al., 1999; Kadyrova et al., 2007) Required for transcriptional repression in the newly specified germ cells of *Drosophila* and *C. elegans* (Deshpande et al., 2005; Schaner et al., 2003) Required for the repression of somatic cell fate in *Drosophila* pole cells (Hayashi et al., 2004)
piwi: argonaute protein, associates with piRNAs	Required for GSC maintenance in *Drosophila* and *C. elegans* (Cox et al., 1998; Lin and Spradling, 1997) Required for spermatogenesis in mouse (Carmell et al., 2007; Kuramochi-Miyagawa et al., 2004) Associates with a novel class of small RNAs termed *piwi*-interacting RNAs, or piRNAs (Aravin et al., 2006; Grivna et al., 2006a) Required to repress transposons in the germline of *Drosophila* and mouse (Aravin et al., 2007; Kalmykova et al., 2005; Saito et al., 2006; Vagin et al., 2006) Required for germline development in *Drosophila* (Megosh et al., 2006) Required with interacting 21U-RNAs for germline maintenance in *C. elegans* (Batista et al., 2008; Wang and Reinke, 2008) Functions in epigenetic regulation in *Drosophila* and mouse (Aravin et al., 2008; Brennecke et al., 2008; Brower-Toland et al., 2007; Kuramochi-Miyagawa et al., 2008; Yin and Lin, 2007) Functions as a translational regulator in the mouse germline (Grivna et al., 2006b; Unhavaithaya et al., 2009)
pumilio: translational repressor	Required for GSC maintenance in *Drosophila* and *C. elegans* (Crittenden et al., 2002; Forbes and Lehmann, 1998; Lin and Spradling, 1997) Required for germline development in *Drosophila* and *C. elegans* (Asaoka-Taguchi et al., 1999; Parisi and Lin, 1999; Subramaniam and Seydoux, 1999)
tudor: protein binding	Required for germline development in *Drosophila* (Boswell and Mahowald, 1985; Thomson and Lasko, 2004) Required for spermatogenesis in mouse (Chuma et al., 2006)
boule/DAZ: RNA-binding protein	Required for germline development in *Xenopus*, human and mouse (Houston and King, 2000; Kee et al., 2009; Lin and Page, 2005) Required for gametogenesis in *Drosophila*, *C. elegans*, human and mouse (Eberhart et al., 1996; Karashima et al., 2000; Reijo et al., 1995; Ruggiu et al., 1997)
germ cell-less: nuclear pore associated protein	Required for germline development in *Drosophila* (Jongens et al., 1994; Jongens et al., 1992) Required for transcriptional repression in *Drosophila* pole cells (Leatherman et al., 2002) Required for spermatogenesis in mouse (Kimura et al., 2003; Leatherman et al., 2000)
Maelstrom: unknown function	Required for spermatogenesis and transposon repression in mouse (Soper et al., 2008) Required for DNA damage checkpoint activation and transposon repression in *Drosophila* (Klattenhoff et al., 2007; Lim and Kai, 2007)
bruno: translational repressor	Translationally represses the germline nucleating gene *oskar* in *Drosophila* (Kim-Ha et al., 1995) mRNA is localized to zebrafish nuage (Hashimoto et al., 2006)

Juliano et al 2010

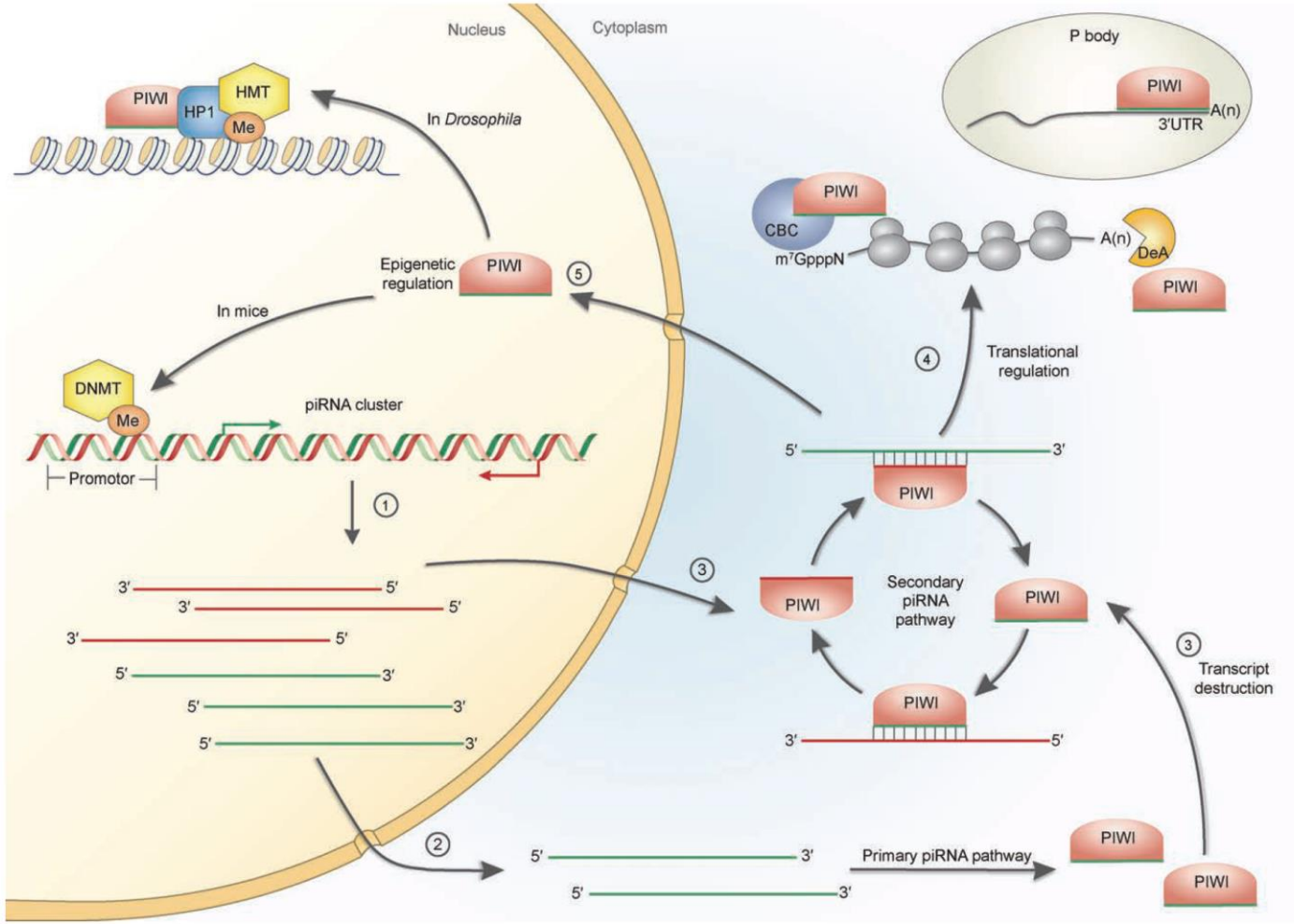


Figure 2. Known gene regulation mechanisms mediated by the PIWI–piRNA pathway. PIWI proteins and piRNAs regulate the expression of genes and transposon at both transcriptional and post-transcriptional levels. (1) Sense and antisense piRNA precursor transcripts are produced in the nucleus. (2) Antisense piRNA precursor transcripts are transported to the cytoplasm and processed by the primary biogenesis pathway to generate mature sense piRNAs that associate with PIWI proteins. (3) The PIWI–antisense piRNA complexes mediate cleavage of sense piRNA precursors and transposon (and protein-coding) transcripts, which silences transposon and gene expression at the post-transcriptional level. The resulting sense transcripts are taken up by PIWI proteins responsible for sense piRNA binding. The PIWI–sense piRNA complexes then cleave antisense piRNA precursor transcripts to amplify the piRNA biogenesis cycle [48,50]. (4) The PIWI–piRNA complexes are involved in translational regulations by interacting with polysomes, mRNA cap-binding complex (CBC, in mice) [7,74,94], and mRNA deadenylase (DeA, in *Drosophila*) [133]. In addition, the PIWI–piRNA complexes are associated with P-body components [37,109,136,137] and piRNAs are mapped to the 3′UTR of mRNAs [131,132]. (5) The PIWI–piRNA complexes can enter the nucleus and regulate gene transcription through epigenetic mechanisms including heterochromatin formation [9,11,114–117] and DNA methylation [10,120] in the promoter region of target genes. 3′UTR, 3′ untranslated region; CBC, cap-binding complex; DeA, deadenylase; DNMT, DNA methyltransferase; HMT, histone methyltransferase; HP1, heterochromatin protein 1; Me, methylation.

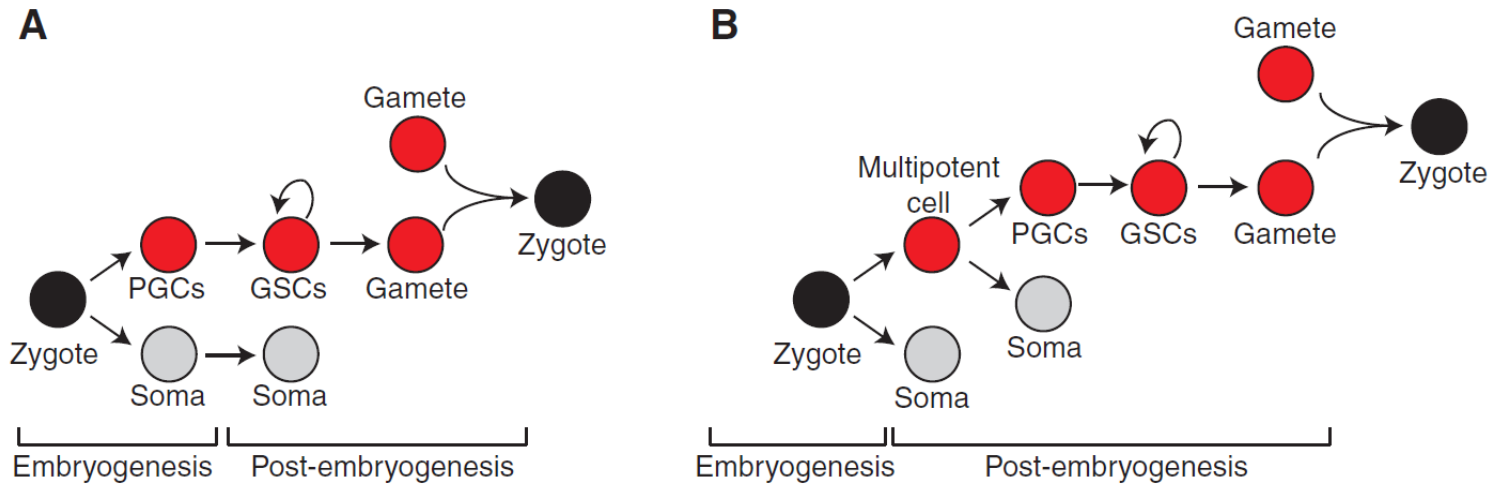
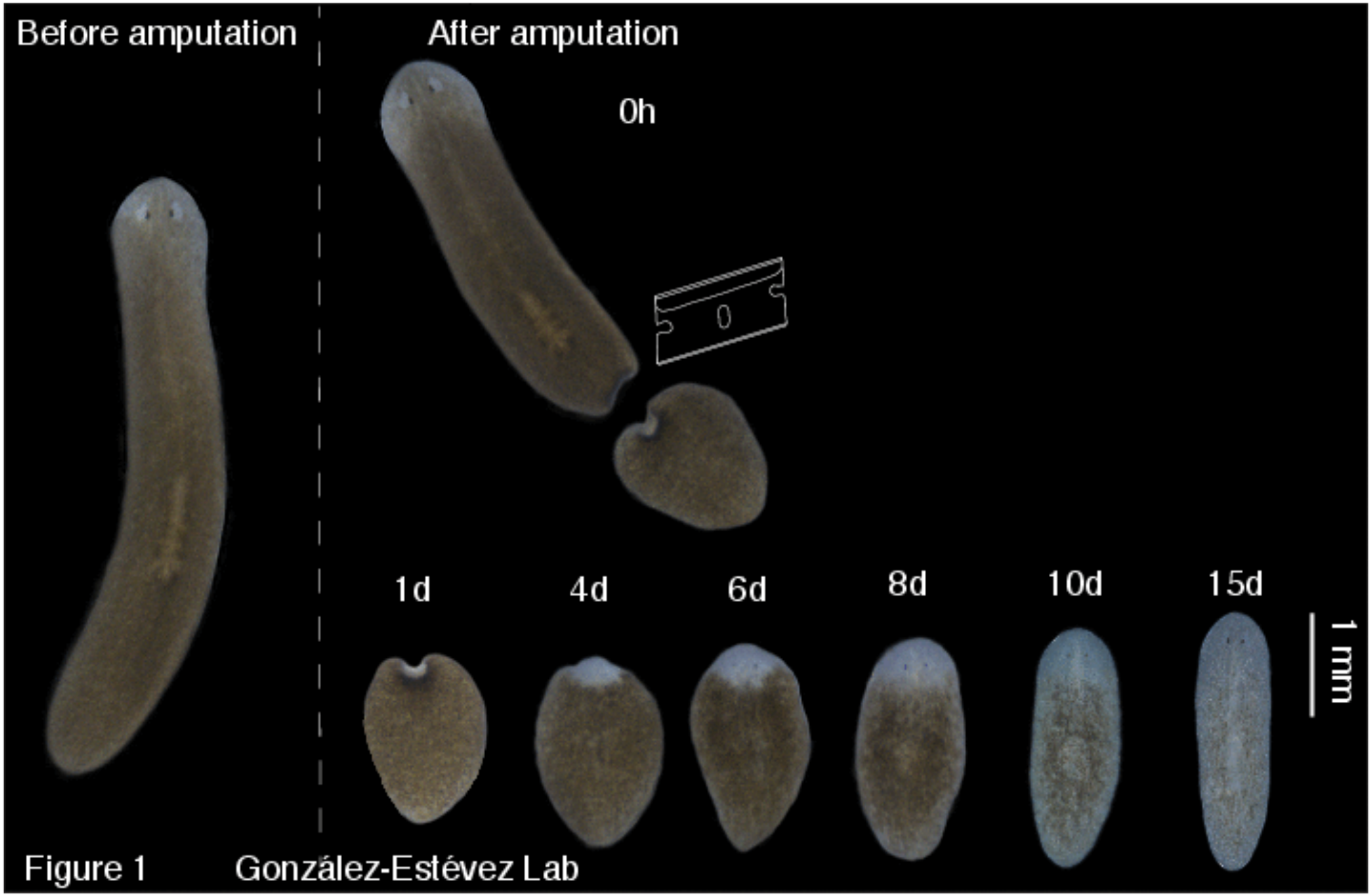


Fig. 1. Germline segregation strategies. (**A**) In animals that segregate their germline during embryogenesis, primordial germ cells (PGCs) are specified in the embryo. PGCs give rise exclusively to either male or female germline stem cells (GSCs), which both self-renew (as indicated by the curved arrows) and give rise to a constant supply of gametes. Gametes are highly specialized cells, but when they fuse at fertilization they create a totipotent zygote. (**B**) In animals that segregate their germline after embryogenesis, a multipotent progenitor is established in the embryo from which the germline is segregated after embryogenesis is completed. We propose that the red cells in both panels operate a conserved germline multipotency program.


Before amputation
After amputation
0h
1d
4d
6d
8d
10d
15d
1 mm
Figure 1
González-Estévez Lab

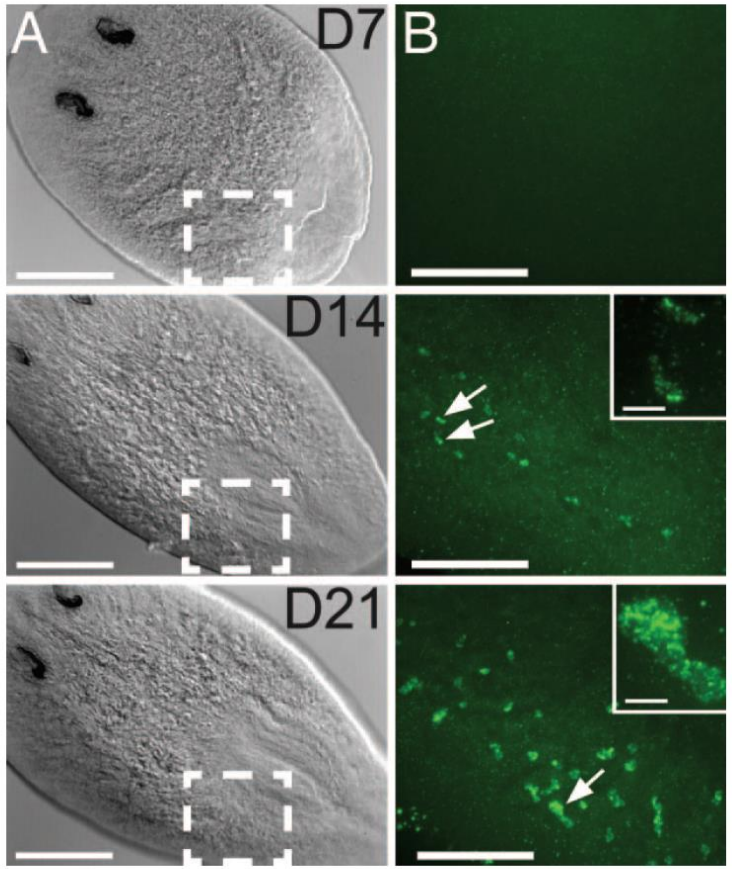
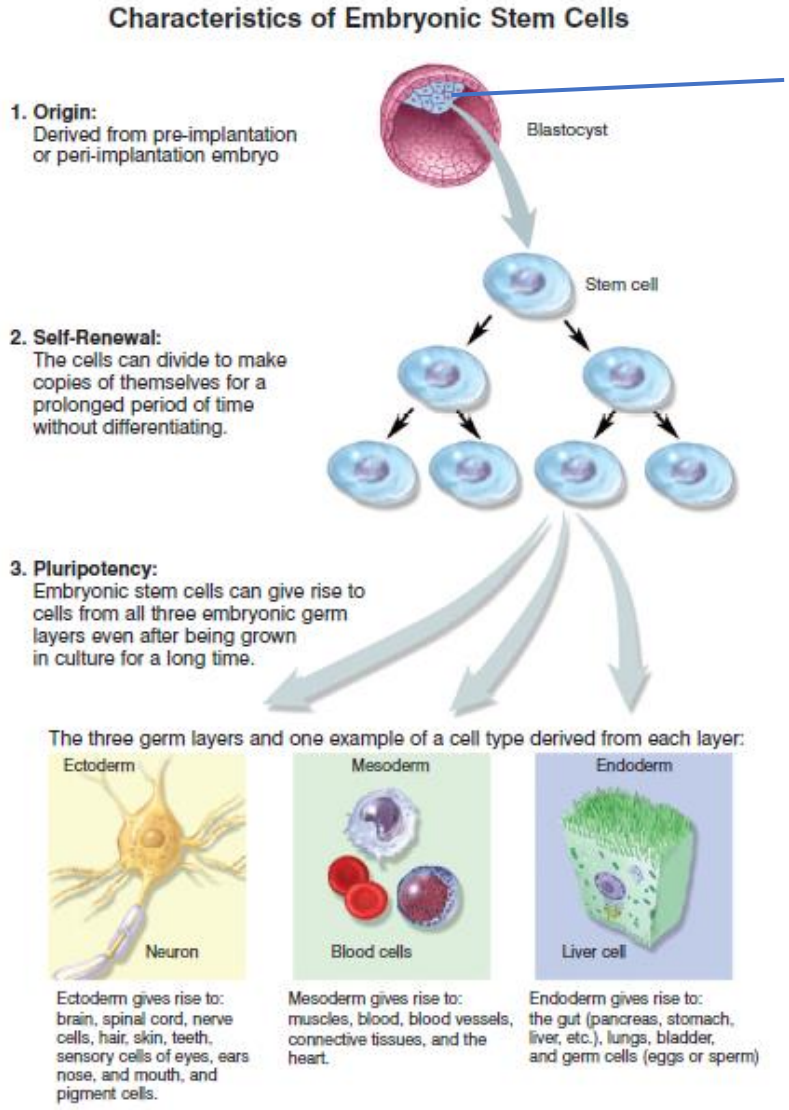


Fig. 2. *Smed-nanos* expression in regenerating head fragments amputated anterior to the ovaries. (*A*) Differential interference contrast microscopic images of regenerating heads fixed 7, 14, or 21 days after amputation (animals were ≥1.2 cm when amputated). The numbers of animals in which *nanos* mRNA was detected were 7 of 14 at 7 days, 8 of 8 at 14 days, and 8 of 9 at 21 days. (Scale bars, 250 μm.) (*B*) Confocal projections corresponding to the boxed regions in A showing *nanos* mRNA detected by FISH. (Scale bars, 100 μm.) Arrows indicate *nanos*-positive cells shown at higher magnification in the *Insets*. (*Inset* scale bars, 10 μm.)

Wang et al 2007

Células madre

Diferenciación y potencialidad
Las células madre embrionarias como ejemplo



ICM – Inner cell mass, dará todos los tejidos embrionarios

Embryonic Stem Cells = ESC
Células Madre Embrionarias

Derivadas del ICM *in vitro*

ESCs tienen programa de expresión génica que les permite renovarse pero al mismo tiempo se encuentran "cebadas" para diferenciarse ante estímulos ambientales adecuados.

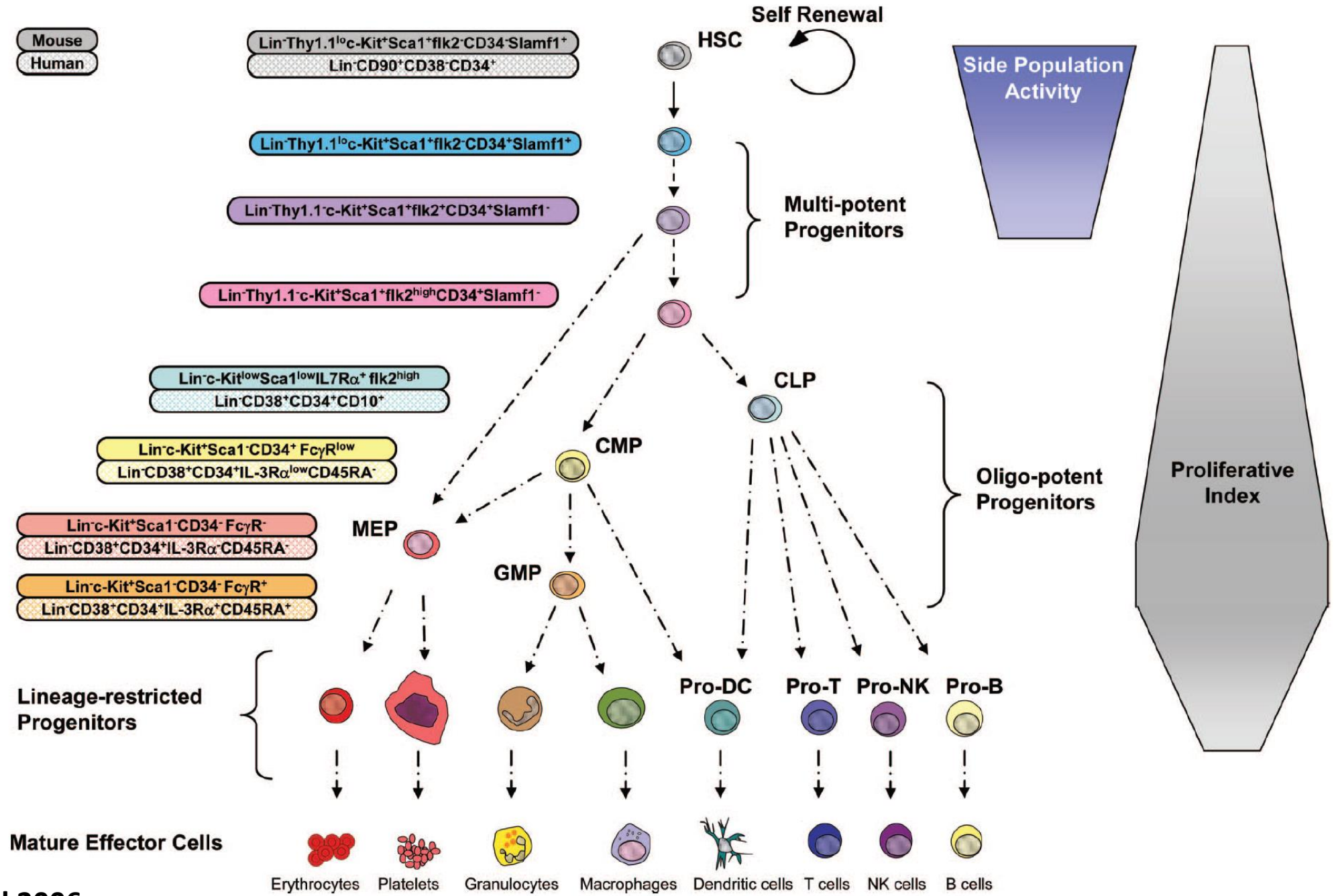
Yu & Thompson, 2006

Células madre en el adulto (Adult Stem Cells)

- Def. potencia auto-renovación
- Def. potencia diferenciación

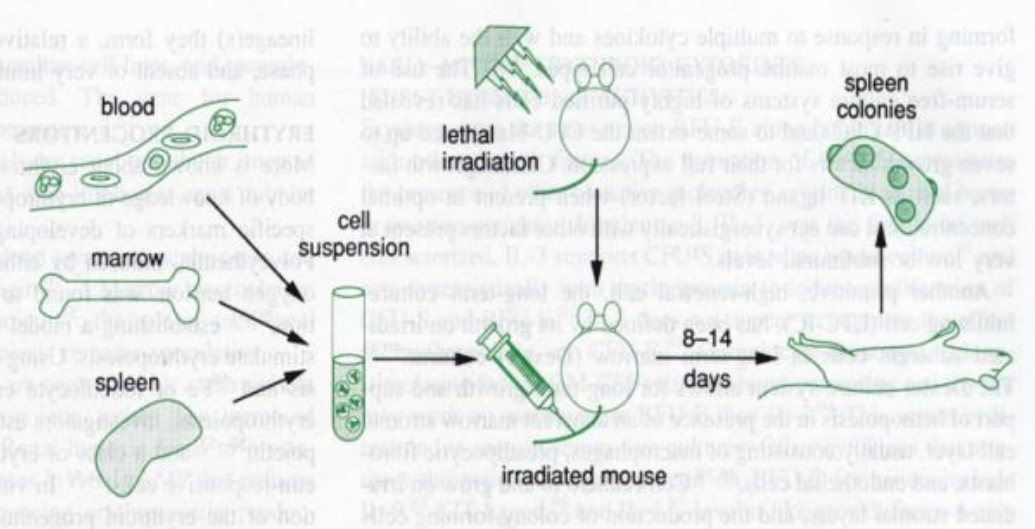
- Muchos ejemplos de células diferenciadas capaces de proliferar: hepatocitos en regeneración, células beta en páncreas

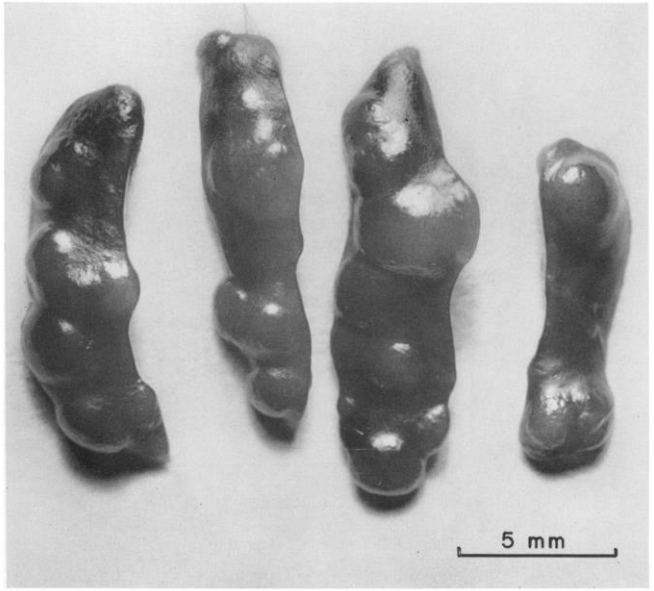
- Ejemplos de células diferenciadas capaces de actuar como células madre



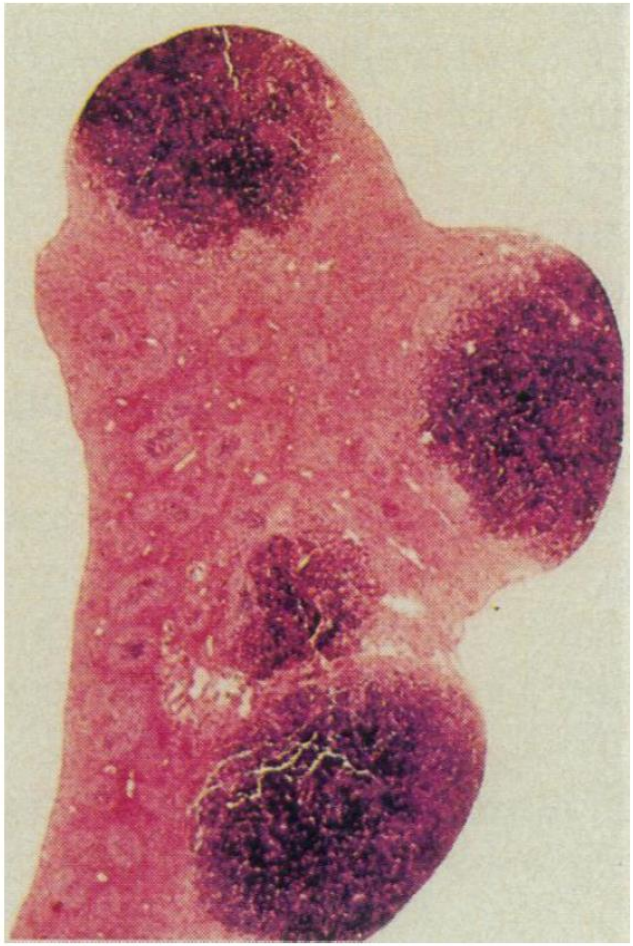
Bryder et al 2006

HSC y origen del concepto de nicho



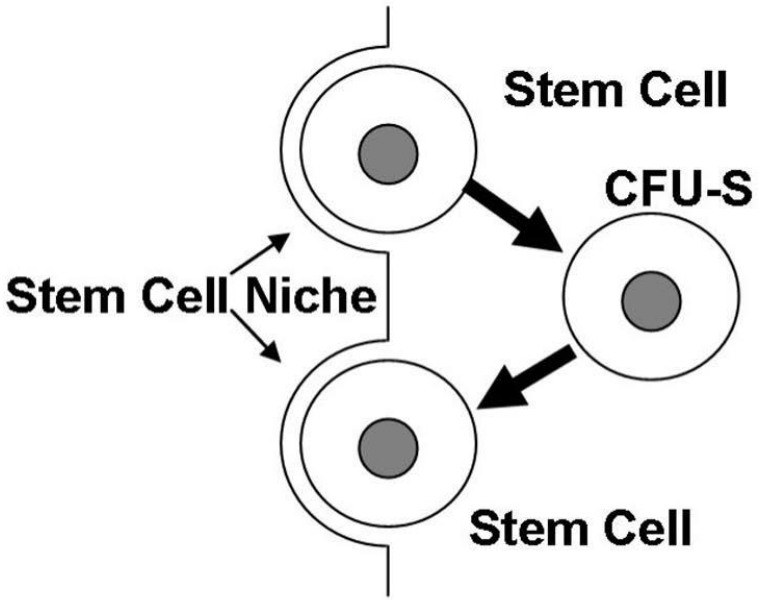


Till & McCulloch 1961



Schofield 1978 – Nicho de HSC

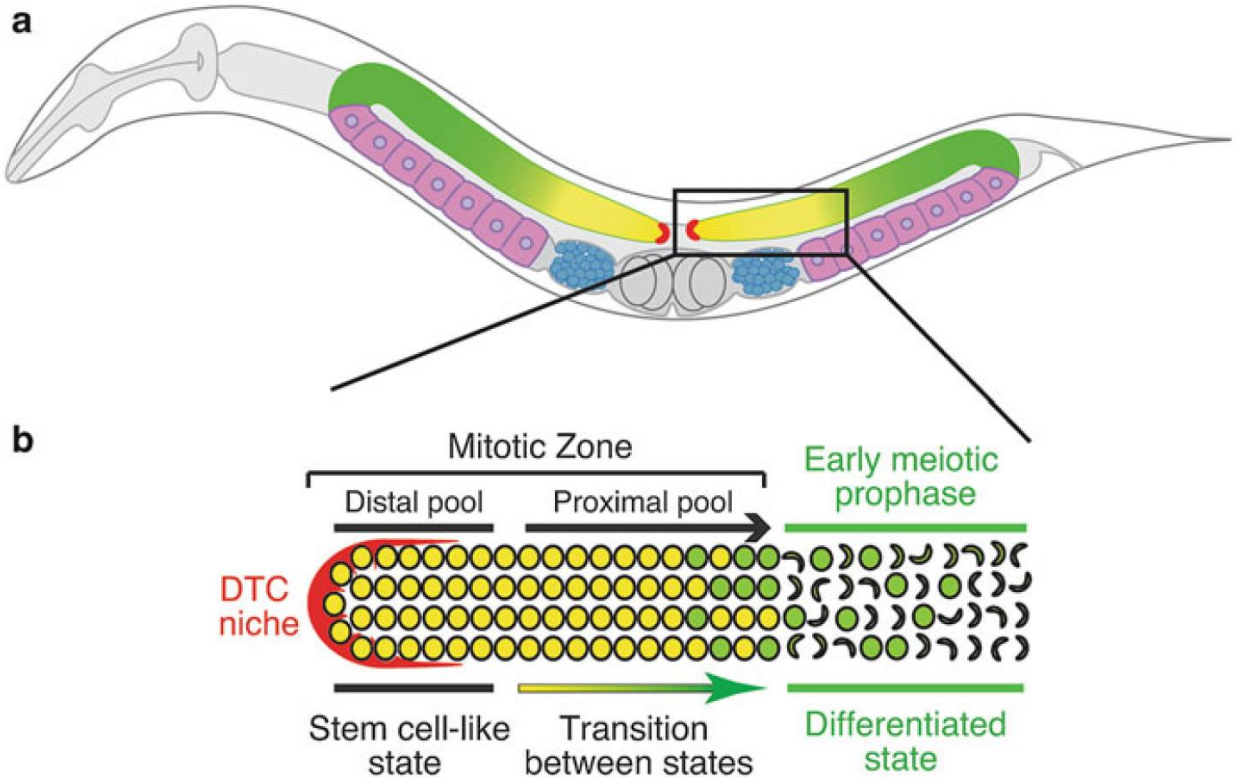
- Células de médula ósea capaces de restaurar hematopoiesis en transplantes seriados
- Pero CFU-S sólo son capaces de restaurar hematopoiesis en 1 – 2 transplantes seriados



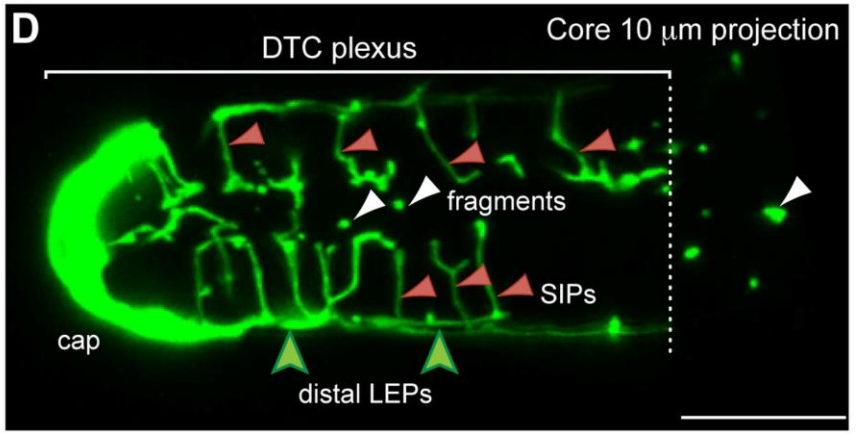
Definición de “nicho”

- (1) a defined anatomic site,
- (2) a location where stem cells could be sustained and reproduce,
- (3) a place where differentiation was inhibited,
- (4) a limited space that also limited the numbers of stem cells
- (5) a place where reversion to a stem-cell phenotype could be induced in a slightly more mature cell type.

C. elegans: células madre germinales



Lag-2::myr-GFP **Byrd et al 2014**



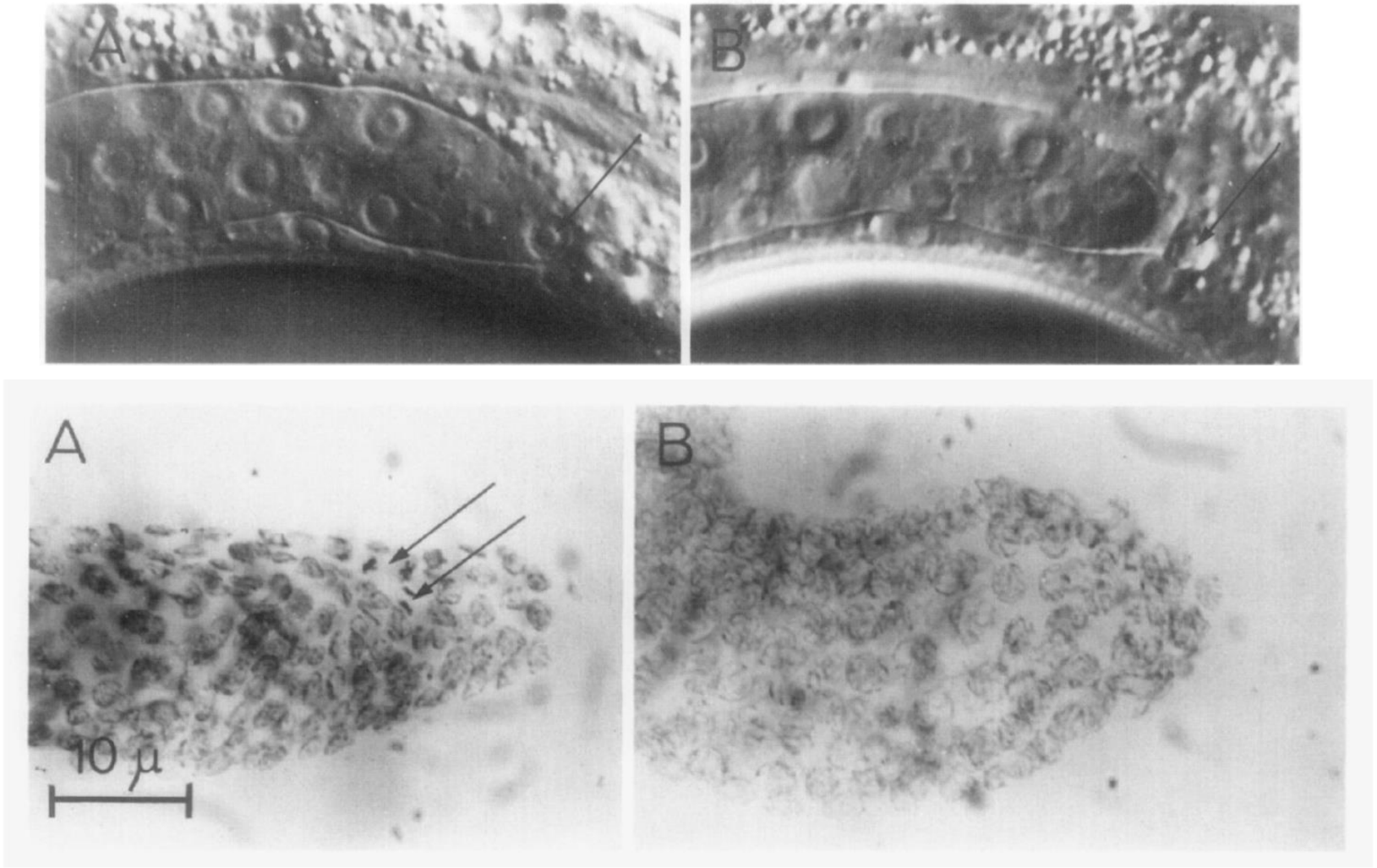
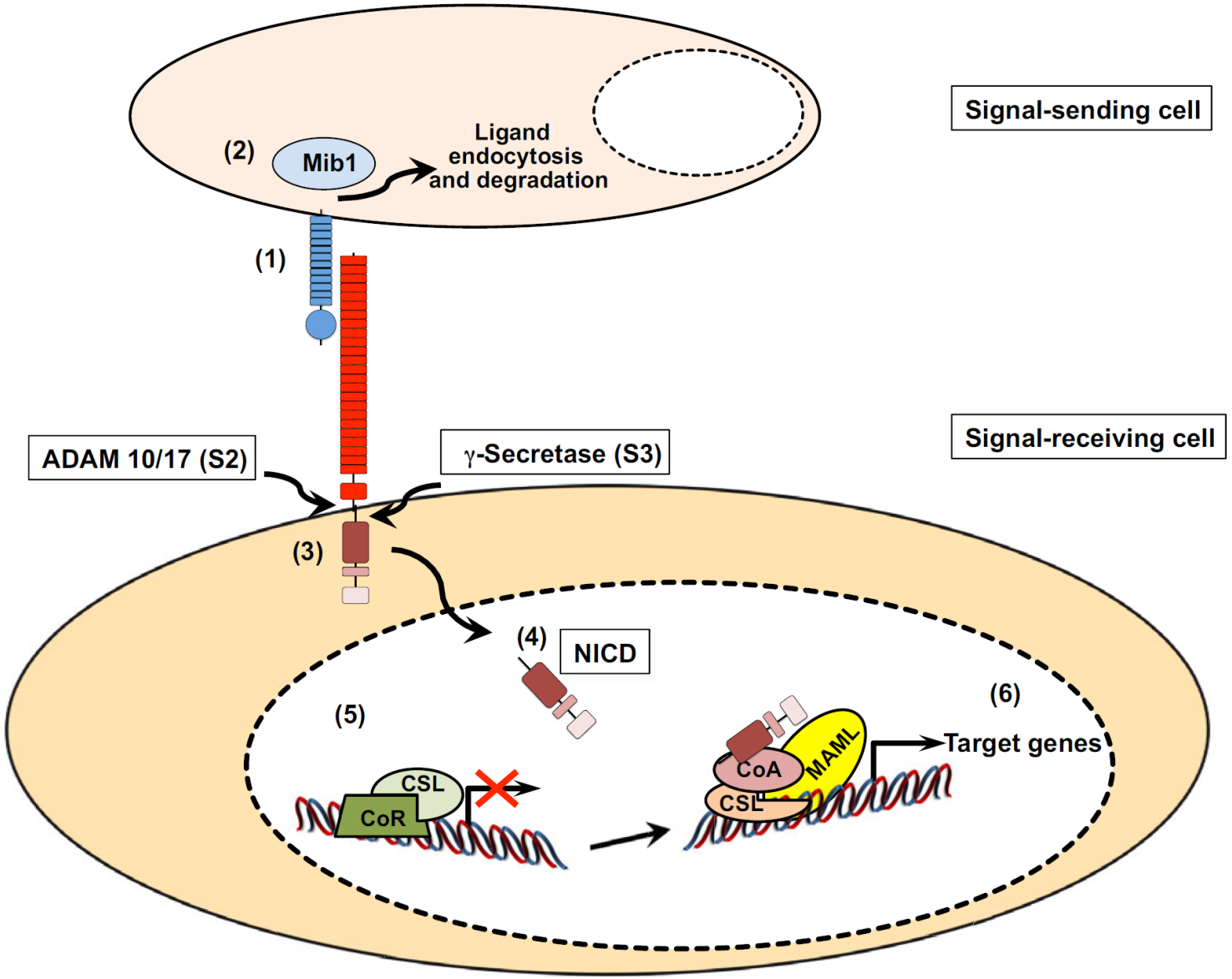


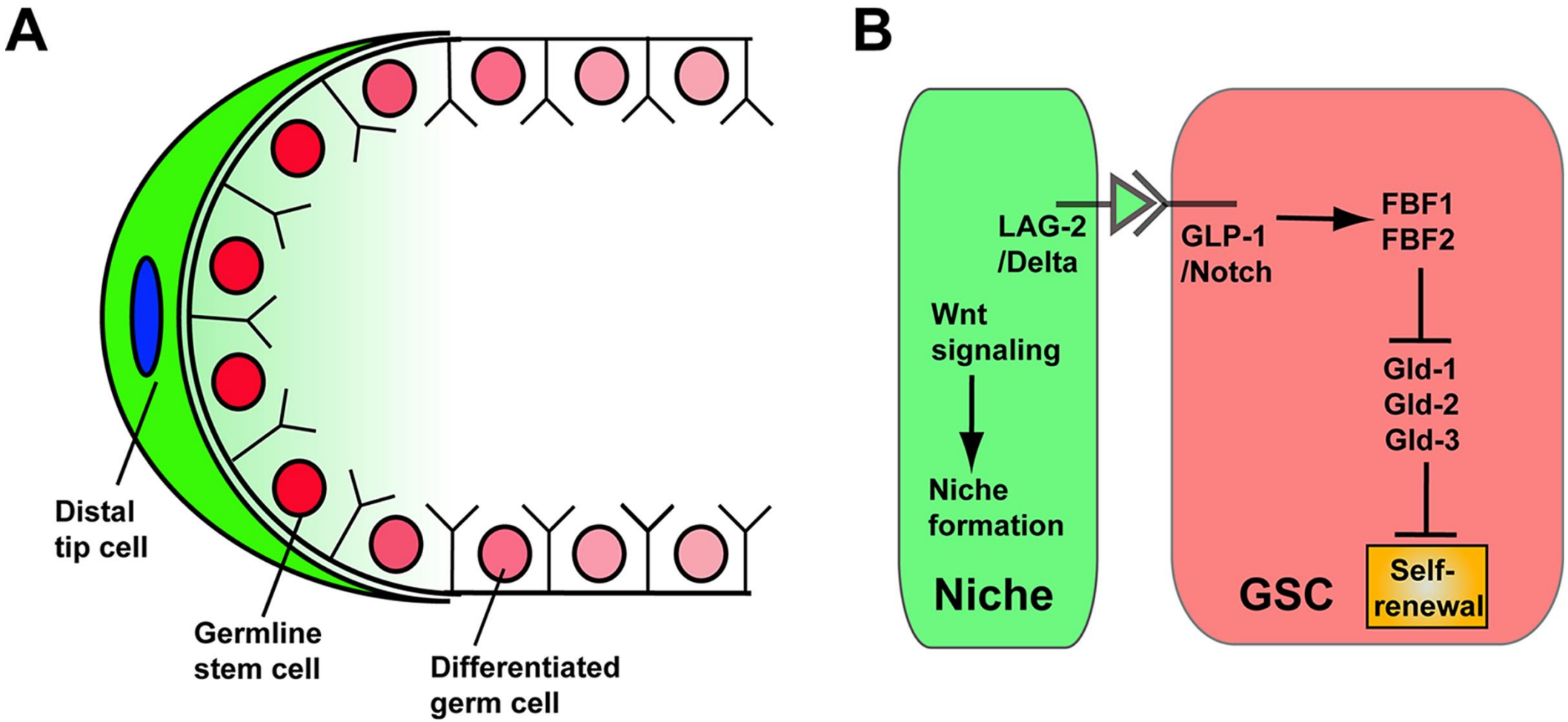
FIG. 5. Chromosome morphology of nuclei before and after distal tip cell ablation observed in Feulgen-stained preparations of dissected gonads. (A) Nuclei in the distal end of an unoperated hermaphrodite gonad are not meiotic. Most are in mitotic interphase and two are dividing (arrows). (B) Nuclei in the distal end of a hermaphrodite gonad about 24 hr after killing its distal tip cell are all in pachytene. No mitotic figures are seen.

Kimble & White 1981

Delta/Notch

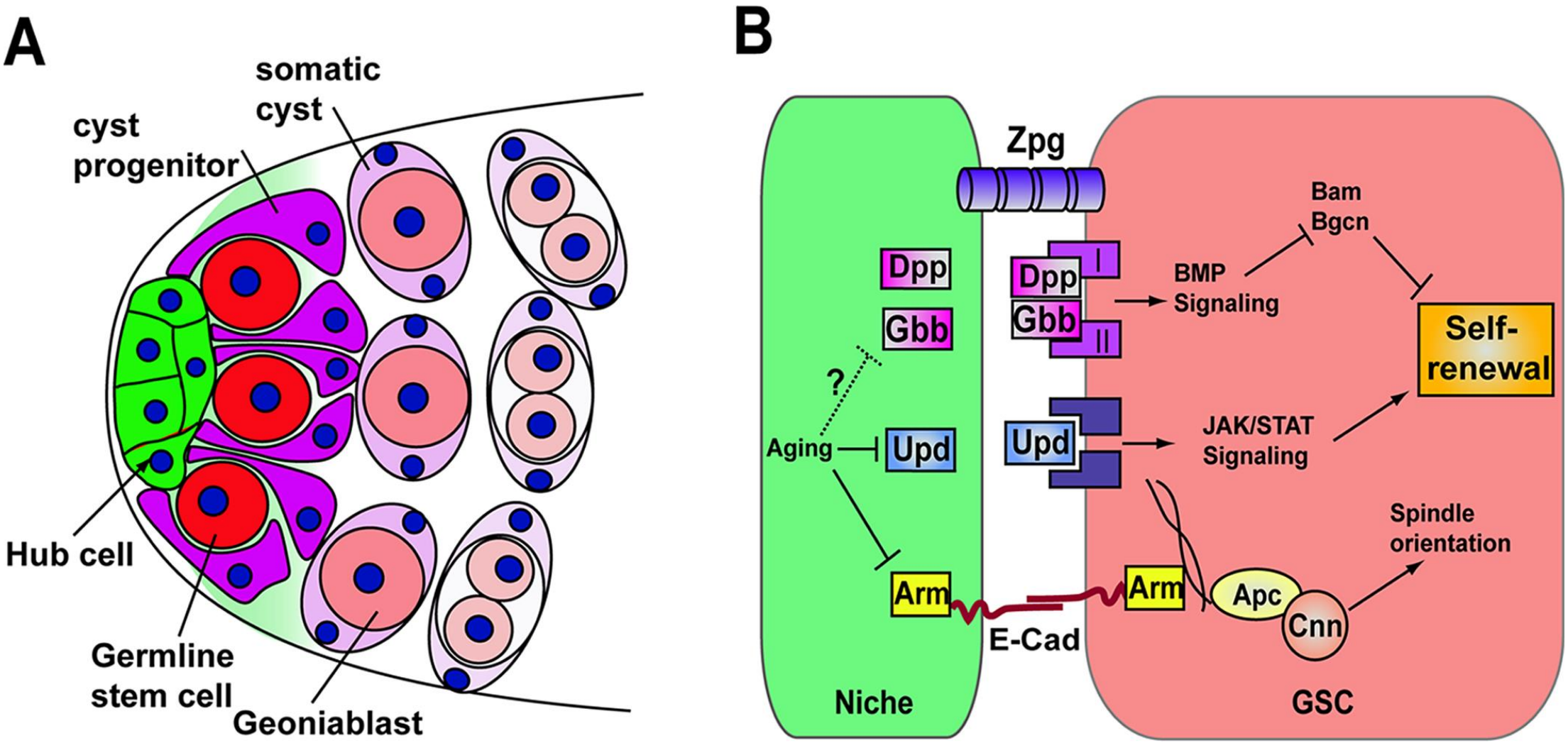


Koch et al 2013


A
Distal
tip cell
Germline
stem cell
Differentiated
germ cell
B
LAG-2
/Delta
Wnt
signaling
Niche
formation
Niche
GLP-1
/Notch
FBF1
FBF2
Gld-1
Gld-2
Gld-3
GSC
Self-
renewal

Xie 2008

D. melanogaster: células madre germinales



Xie 2008

JAK/STAT

(Unpaired)

(Hopscotch)

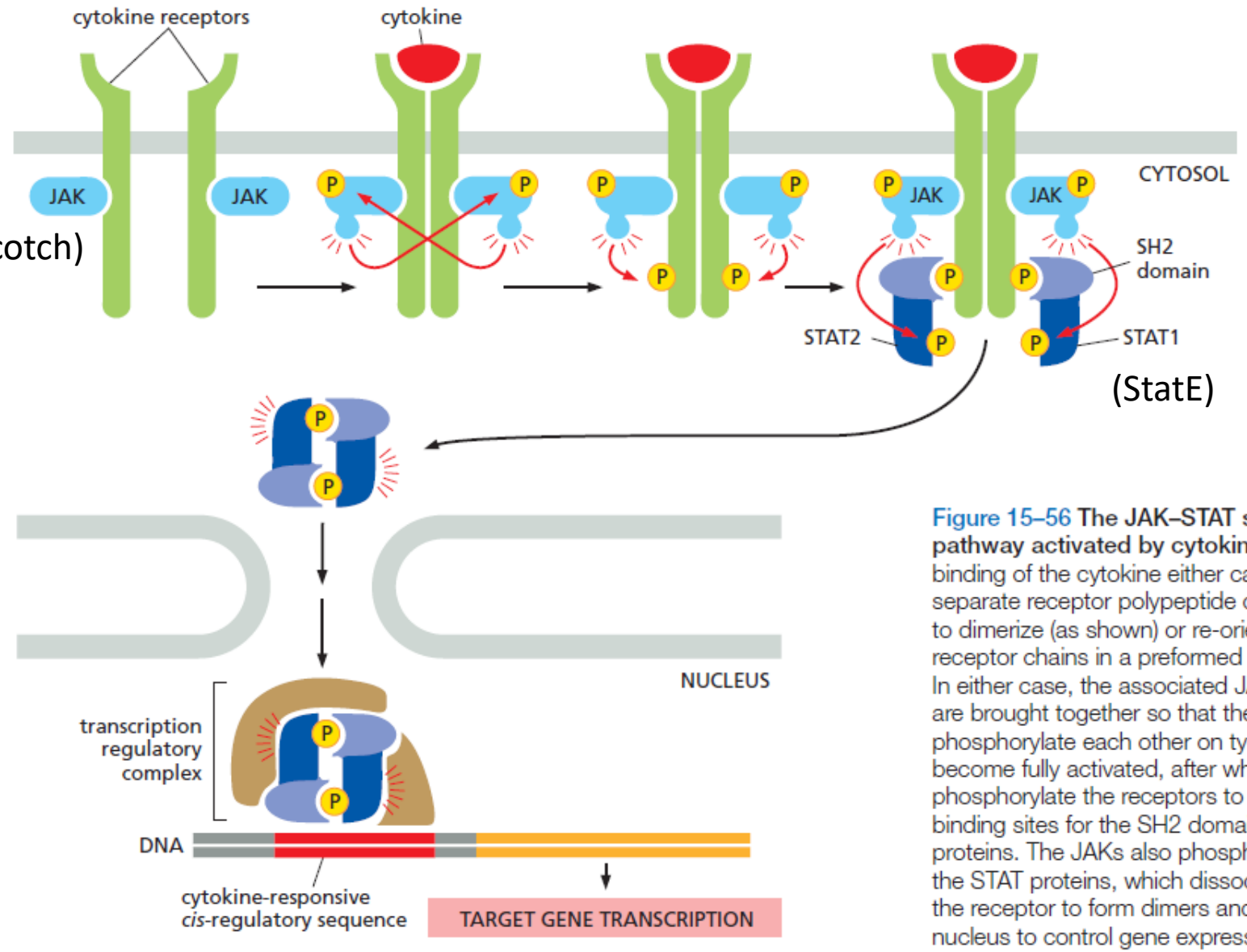
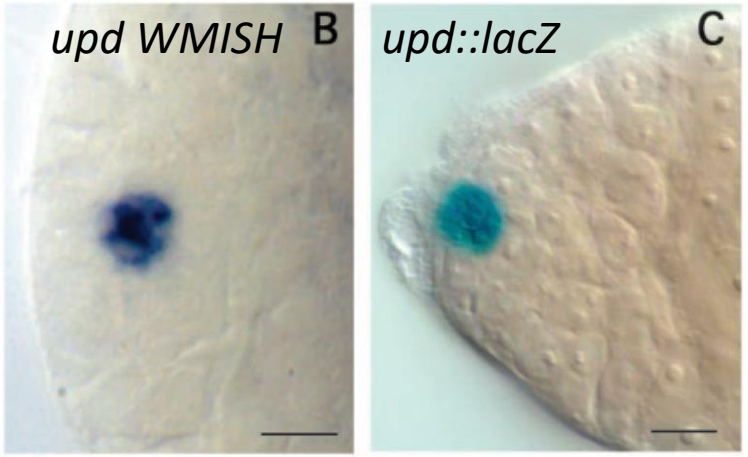
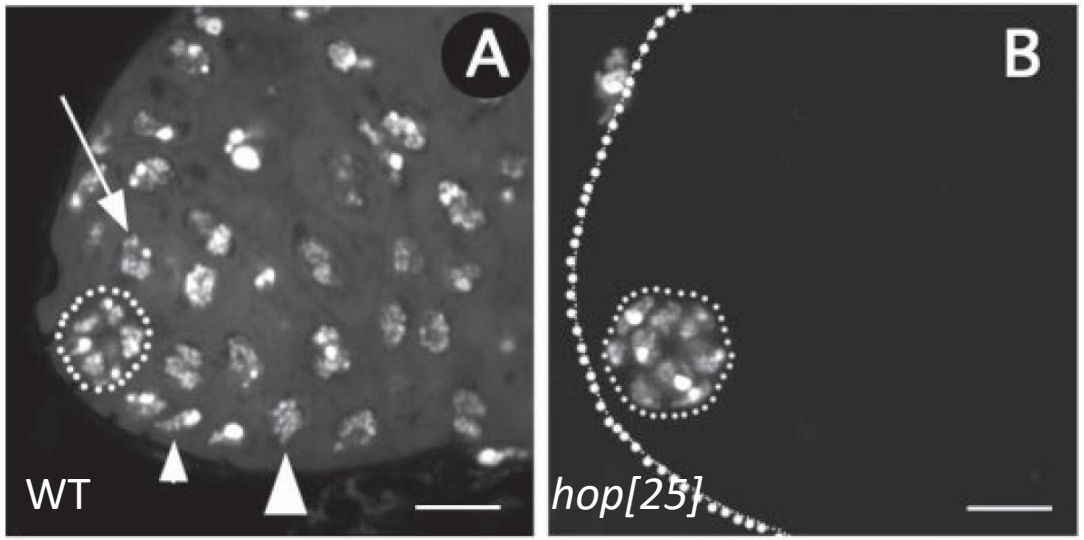


Figure 15–56 The JAK–STAT signaling pathway activated by cytokines. The binding of the cytokine either causes two separate receptor polypeptide chains to dimerize (as shown) or re-orients the receptor chains in a preformed dimer. In either case, the associated JAKs are brought together so that they can phosphorylate each other on tyrosines to become fully activated, after which they phosphorylate the receptors to generate binding sites for the SH2 domains of STAT proteins. The JAKs also phosphorylate the STAT proteins, which dissociate from the receptor to form dimers and enter the nucleus to control gene expression.



Unpaired (*upd*) – ligando principal de vía JAK/STAT en *Drosophila*

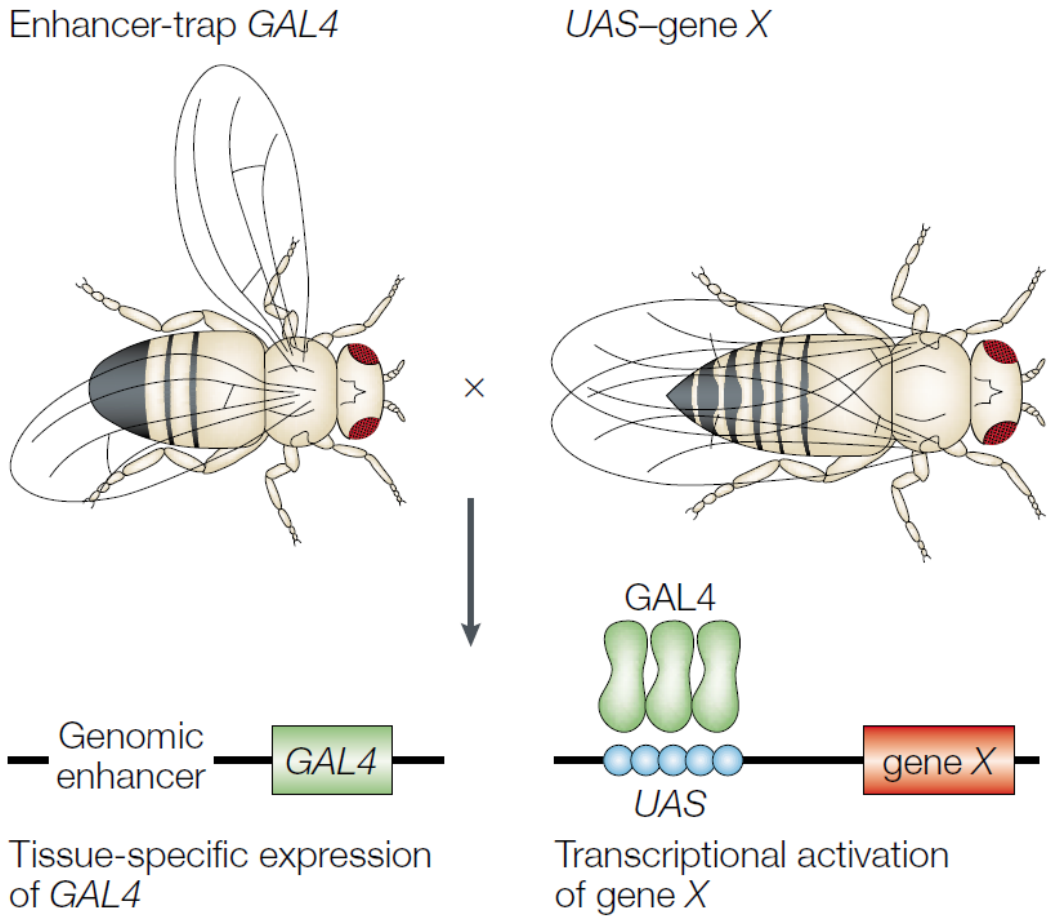


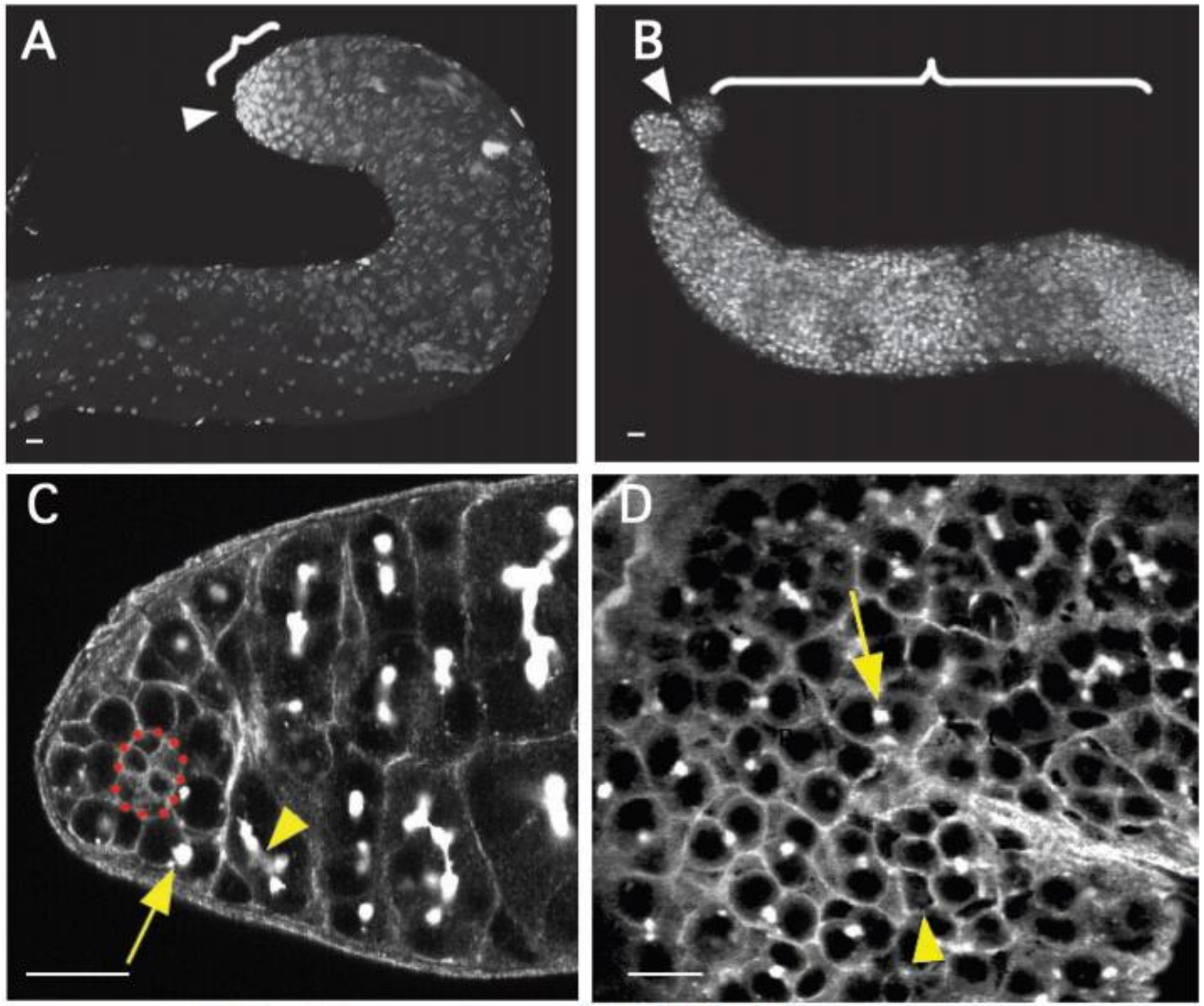
Hopscotch (*hop*) – Homólogo de JAK

Tulina & Matunis 2001

Box 2 | **The *GAL4–UAS* system for directed gene expression**

The yeast transcriptional activator Gal4 can be used to regulate gene expression in *Drosophila* by inserting the upstream activating sequence (*UAS*) to which it binds next to a gene of interest (gene *X*)[96]. The *GAL4* gene has been inserted at random positions in the *Drosophila* genome to generate 'enhancer-trap' lines that express *GAL4* under the control of nearby genomic enhancers, and there is now a large collection of lines that express *GAL4* in a huge variety of cell-type and tissue-specific patterns[97]. Therefore, the expression of gene *X* can be driven in any of these patterns by crossing the appropriate *GAL4* enhancer-trap line to flies that carry the *UAS*–gene *X* transgene. This system has been adapted to carry out genetic screens for genes that give phenotypes when misexpressed in a particular tissue (modular misexpression screens)[79].

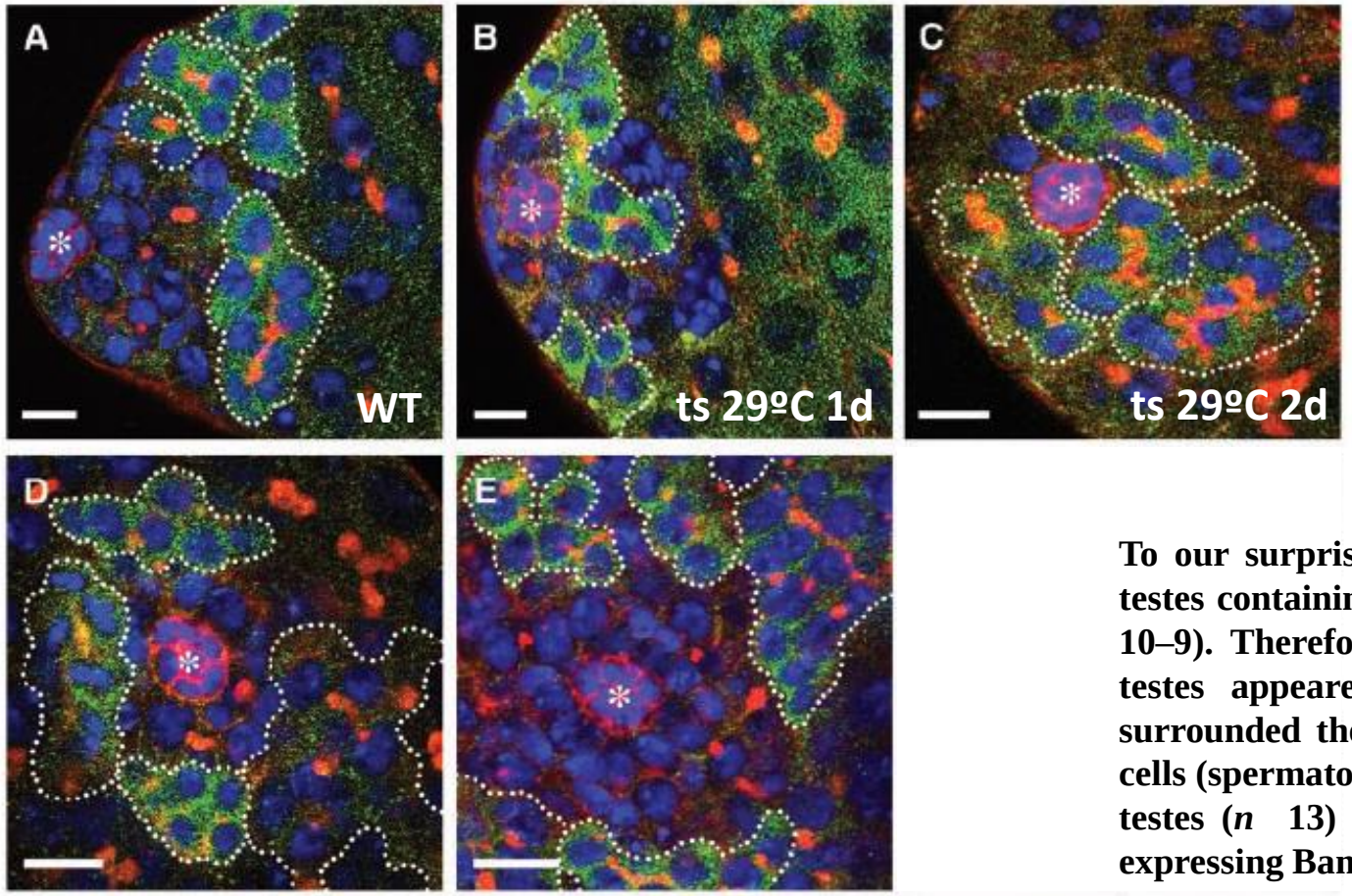




Upd ectópico

P[w1; A4-1Nos-Gal4::VP16nos.UTR]; P[w1, UAS Upd]

Tulina & Matunis 2001



stat92E^{F} **– alelo termosensible**

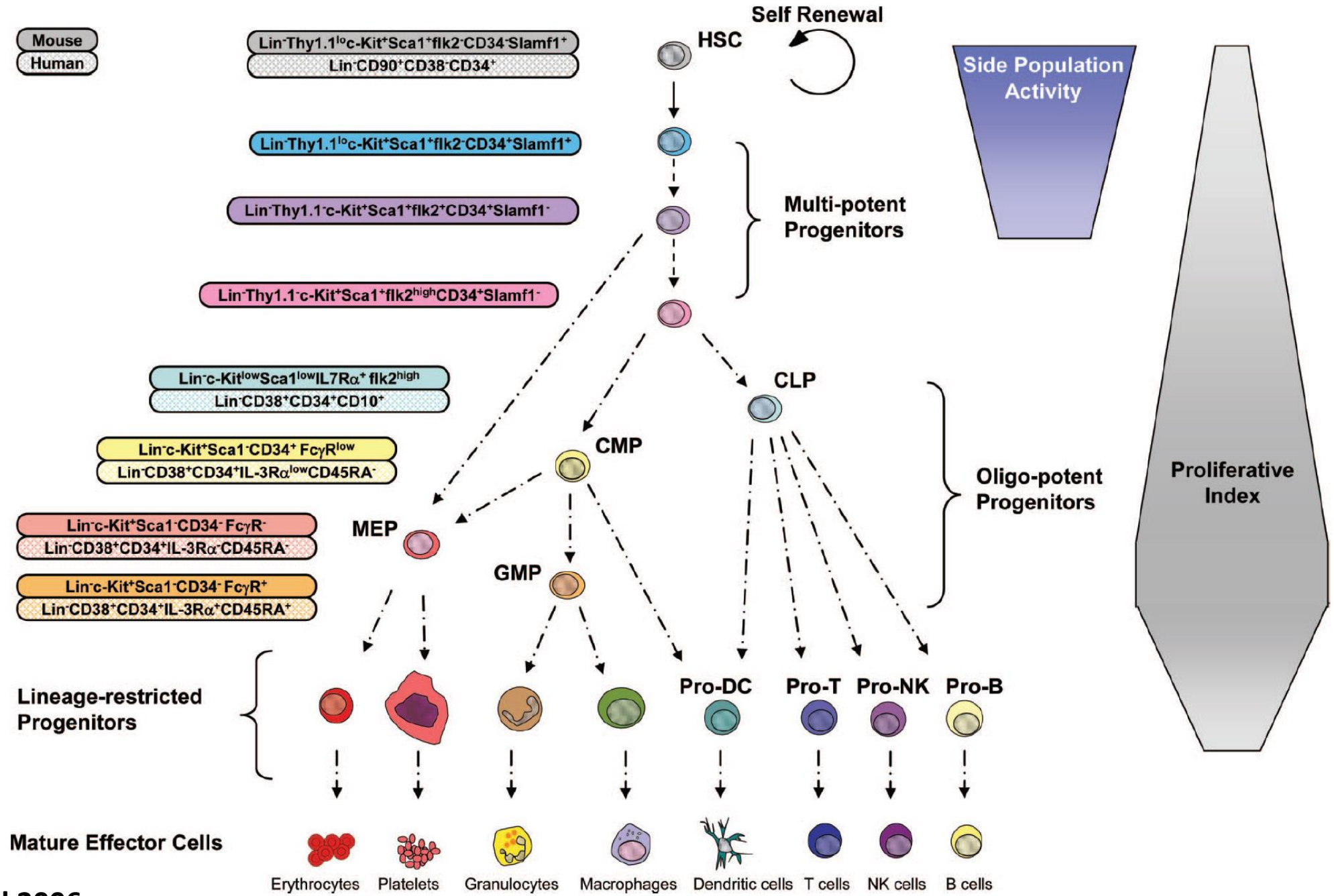
Fusomas
BAM (diferenciación)
DAPI (núcleos)

To our surprise, after a brief recovery at 18°C (2 days), the number of testes containing GSCs increased from 22.5 to 75.8% (*n* 33 testes; *P* 2.29 10–9). Therefore, testes completely lacking GSCs regained GSCs. These testes appeared strikingly different from wild type; although GSCs surrounded the hub, they contacted spermatocytes, because intermediate cells (spermatogonia) were absent. After a longer recovery period, 76.9% of testes (*n* 13) regained a normal zone of spermatogonial cysts (Fig. 2J) expressing Bam (Fig. 2E).

Brawley & Matunis 2004

Como determinar potencialidad de autorenovación y de diferenciación?

- **In vitro**
- **Transplantes**
- **Marcado genético de linajes**



Bryder et al 2006

Diferenciación in vitro de progenitores hematopoyéticos

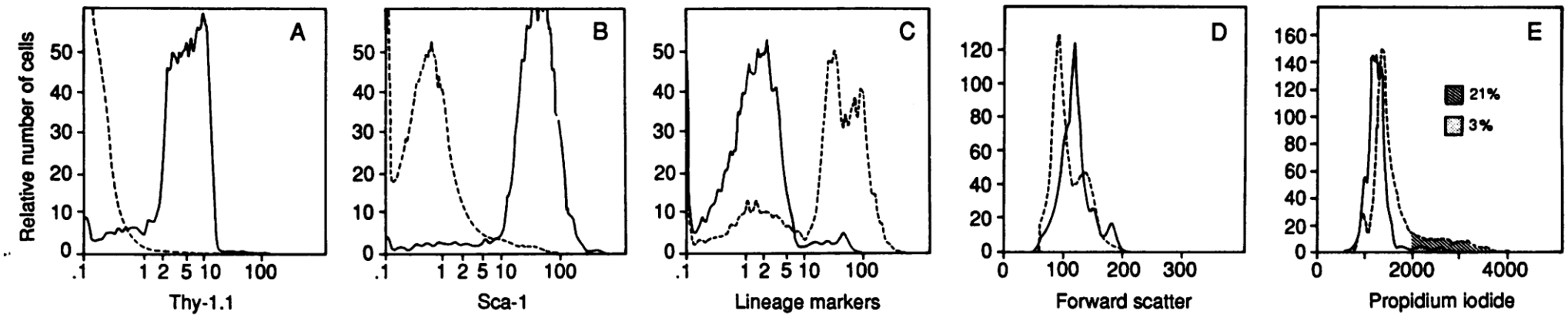
Table 1. Colony formation by fractionated myeloid progenitor cells

	Number of colonies						
Fraction	**Blast**	**G**	**GM**	**M**	**Eo**	**Meg**	**E**
GMP	0	62	22	32	2	0	0
CMP	10	12	16	12	0	3	0
MEP	0.8	0.6	0	0	0	6	2

All cultures contained 100 C57BL-derived marrow cells of each type per 1 milliliter of culture. All cultures were stimulated by 500 ng of stem cell factor + 10 ng interleukin-3 + 2 IU erythropoietin. For a fuller description of the phenotype and how to identify these various colony types, see [21].
Abbreviations: blast, blast cell; CMP, common myeloid progenitors; E, erythroid; Eo, eosinophil; G, granulocyte; GM, granulocyte-macrophage; GMP, granulocyte-macrophage progenitors; M, macrophage; Meg, megakaryocyte; MEP, megakaryocyte-erythroid progenitors.

Metcalf 2007

Transplante de células madre hematopoyéticas



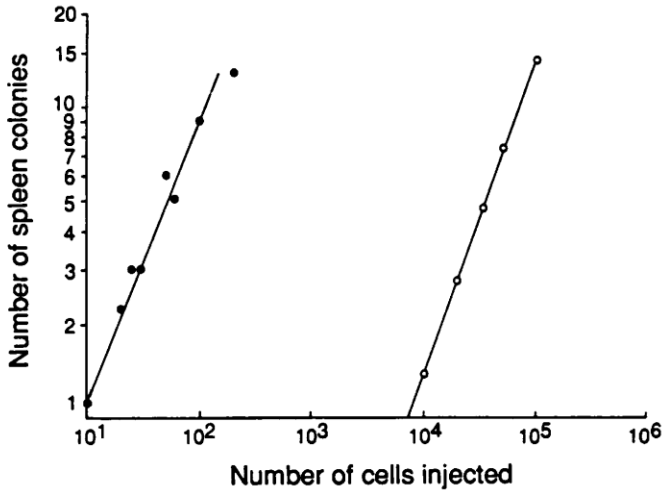


Fig. 2. Spleen colony formation by purified stem cells. Splenic colony-forming unit (CFU-S) activity was assessed 12 days after intravenous transfer of unseparated bone marrow cells or isolated hematopoietic stem cells into lethally irradiated (900 rads) syngeneic mice. Each data point represents the mean from two to four independent experiments with five to ten animals per trial. By linear regression analysis, one splenic colony was formed per ten hematopoietic stem cells transferred [frequency = 0.95 ± 0.08 (SD)], or per 7200 unseparated bone marrow cells [frequency = $(1.4 \pm 0.3) \times 10^{-4}$].

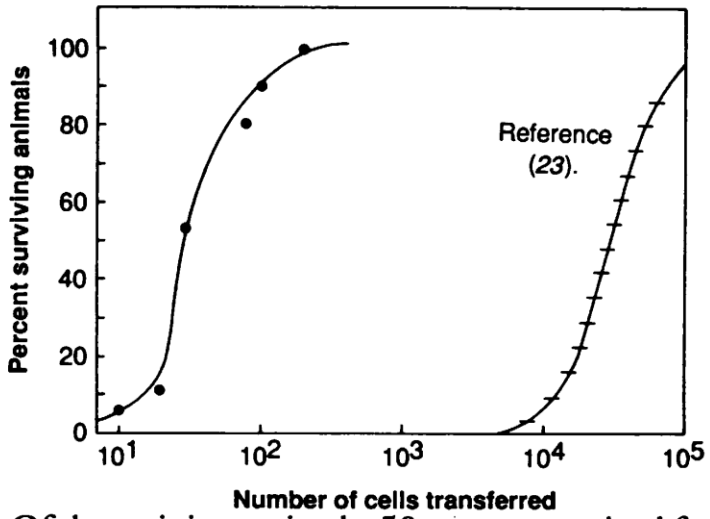
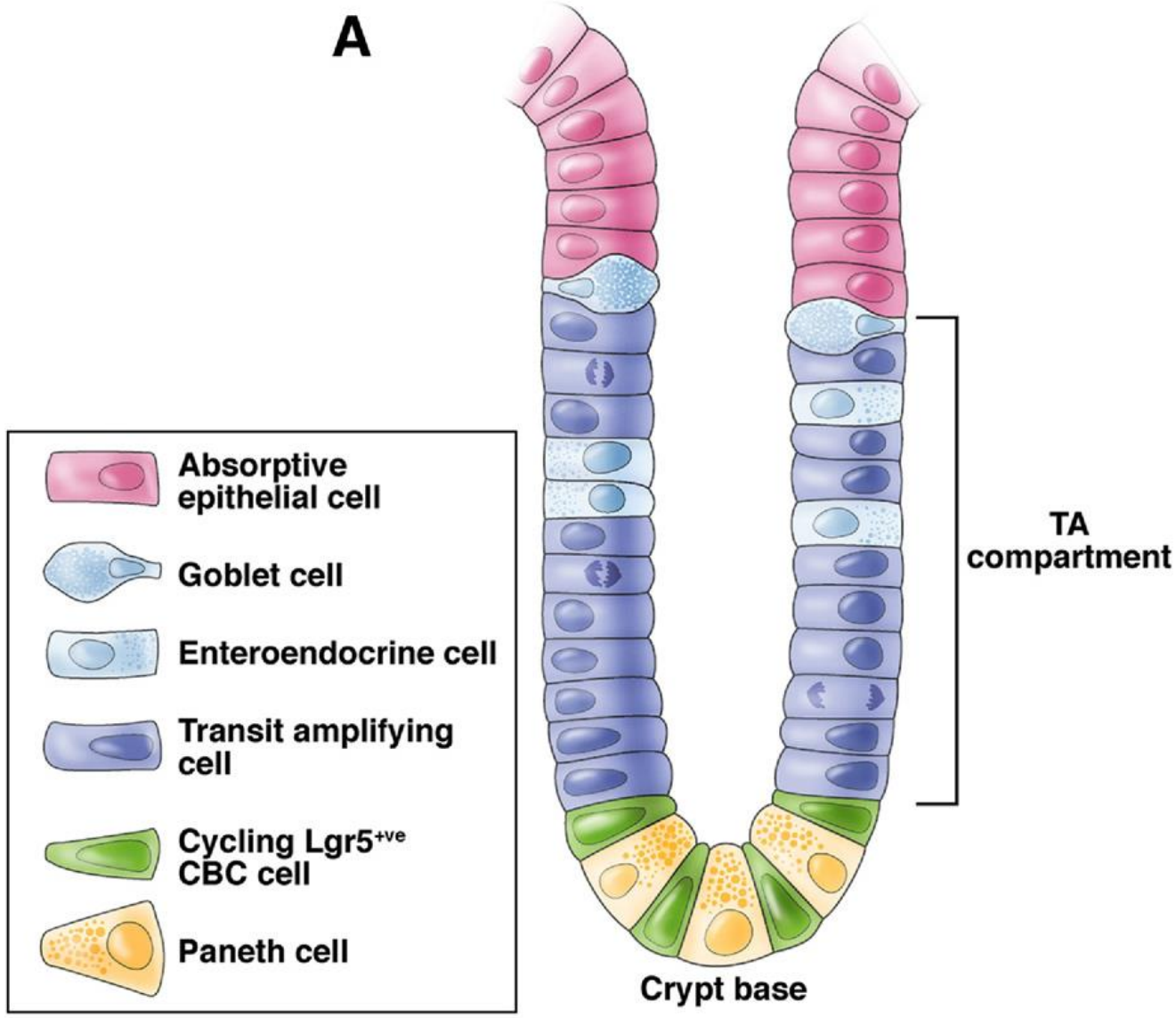


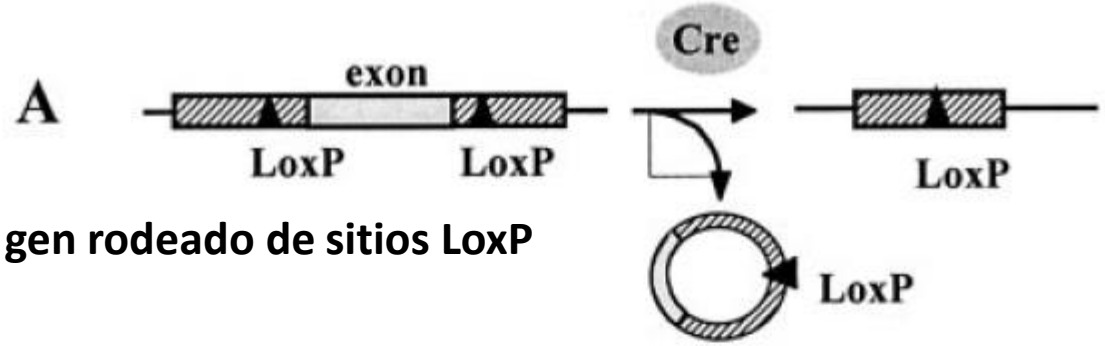
Fig. 5. Protection from lethal irradiation by purified stem cells. Groups of 10 to 20 mice were lethally irradiated (900 rads) and reconstituted with graded numbers of Thy-1^{lo}Lin$^-$Sca-1^+ hematopoietic stem cells intravenously (●). Each point represents the mean from one to two independent experiments, ten animals per trial. Of the recipient animals, 50 percent survived for more than 30 days when 30 cells were transferred. In contrast, we and others have reported that 1.3×10^4 to 3.3×10^4 unseparated bone marrow cells are required to achieve the same level of protection (*6*, *23*); the barred curve is derived from (*23*).

Spangrude et al 1988

Marcado genético de linajes de células madre intestinales



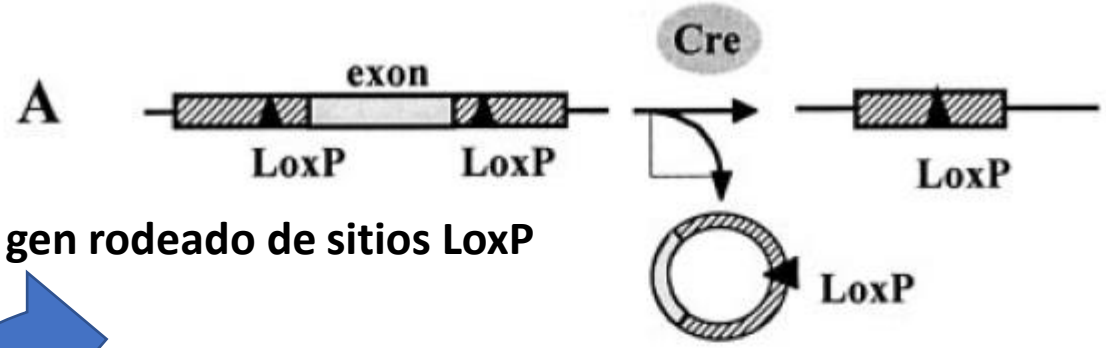
Barker & Clevers 2010



Exón de gen rodeado de sitios LoxP

Cre recombinasa elimina región entre sitios LoxP
Esto ocurre únicamente en las células que expresan Cre

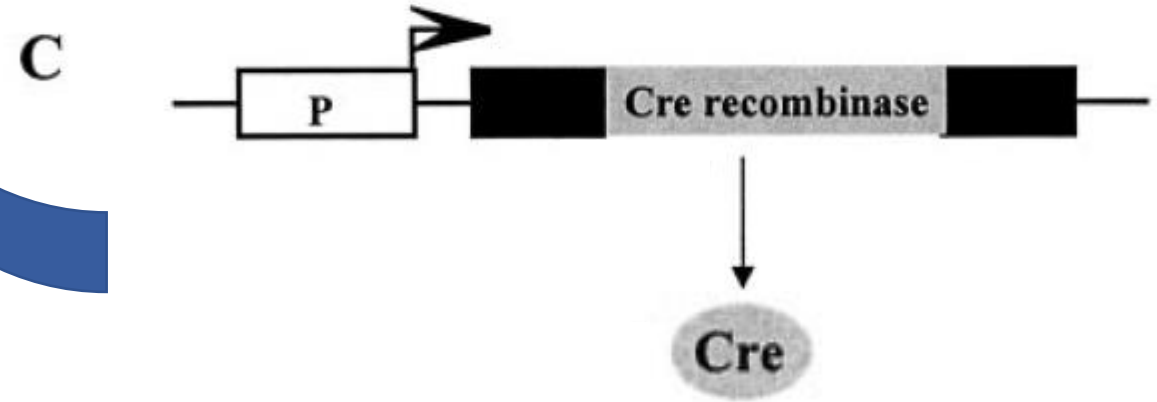
Jaisser 2000 J Am Soc Nephrol 11: S95



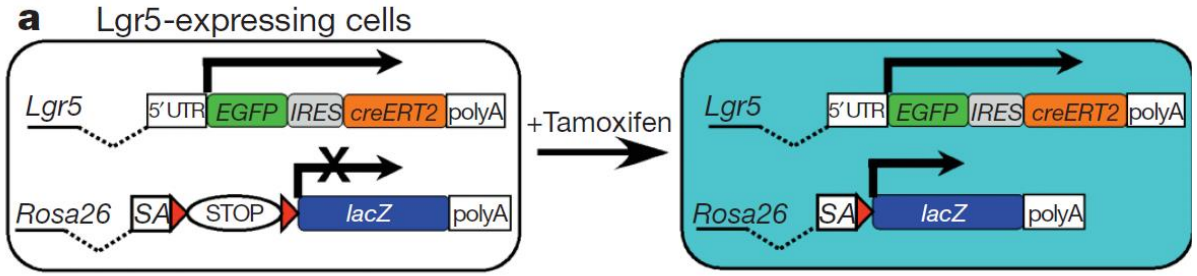
Exón de gen rodeado de sitios LoxP

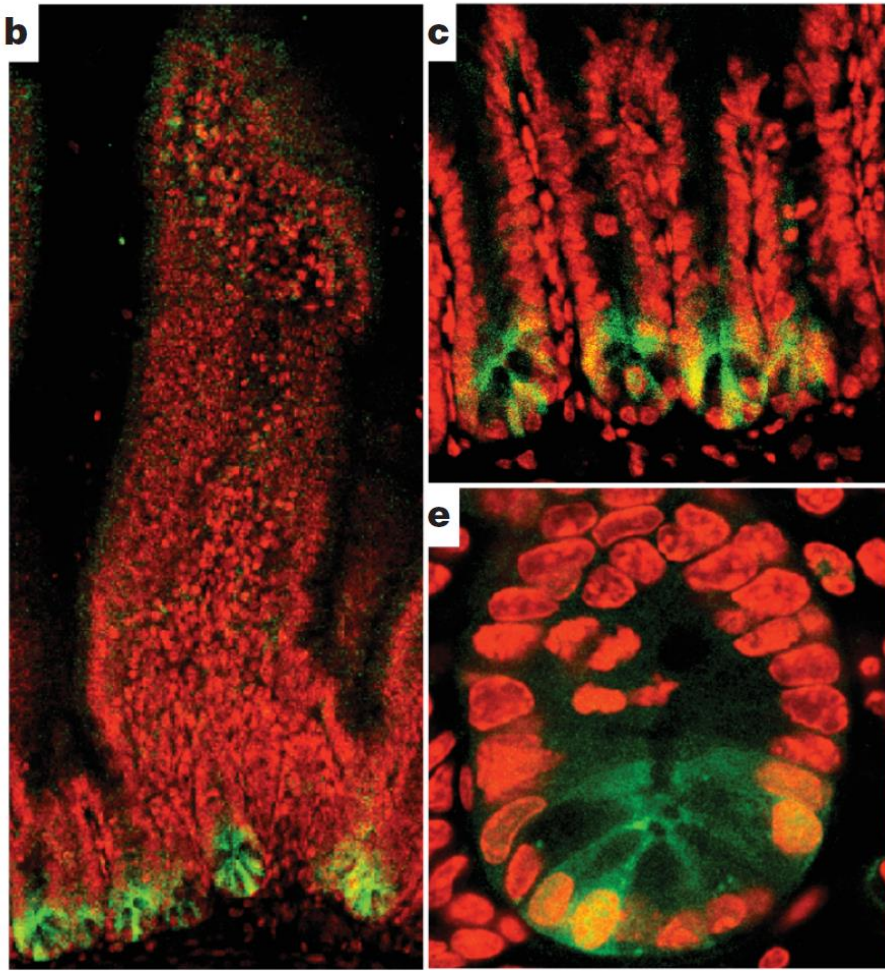
Cre recombinasa elimina región entre sitios LoxP
Esto ocurre únicamente en las células que expresan Cre

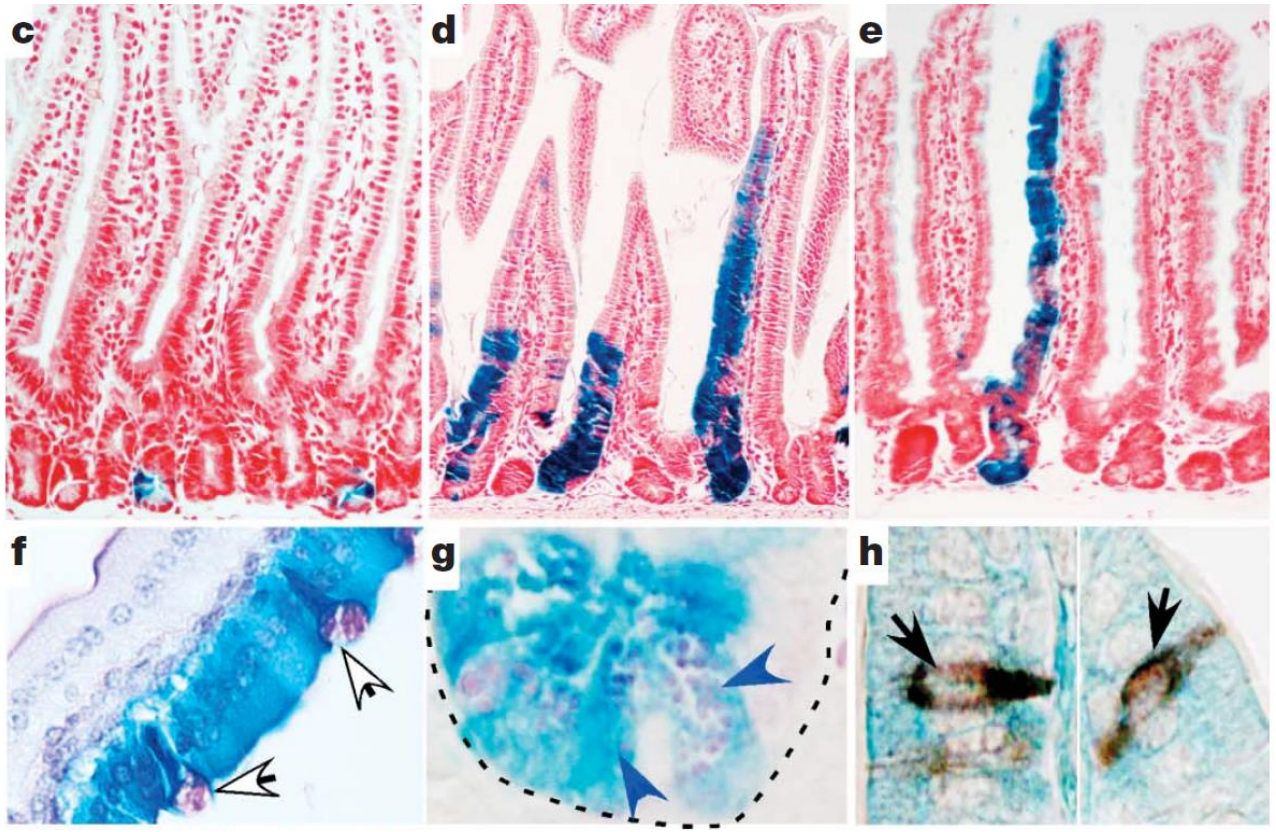
Cre recombinasa bajo control de promotor específico de cierto linaje celular



Jaisser 2000 J Am Soc Nephrol 11: S95

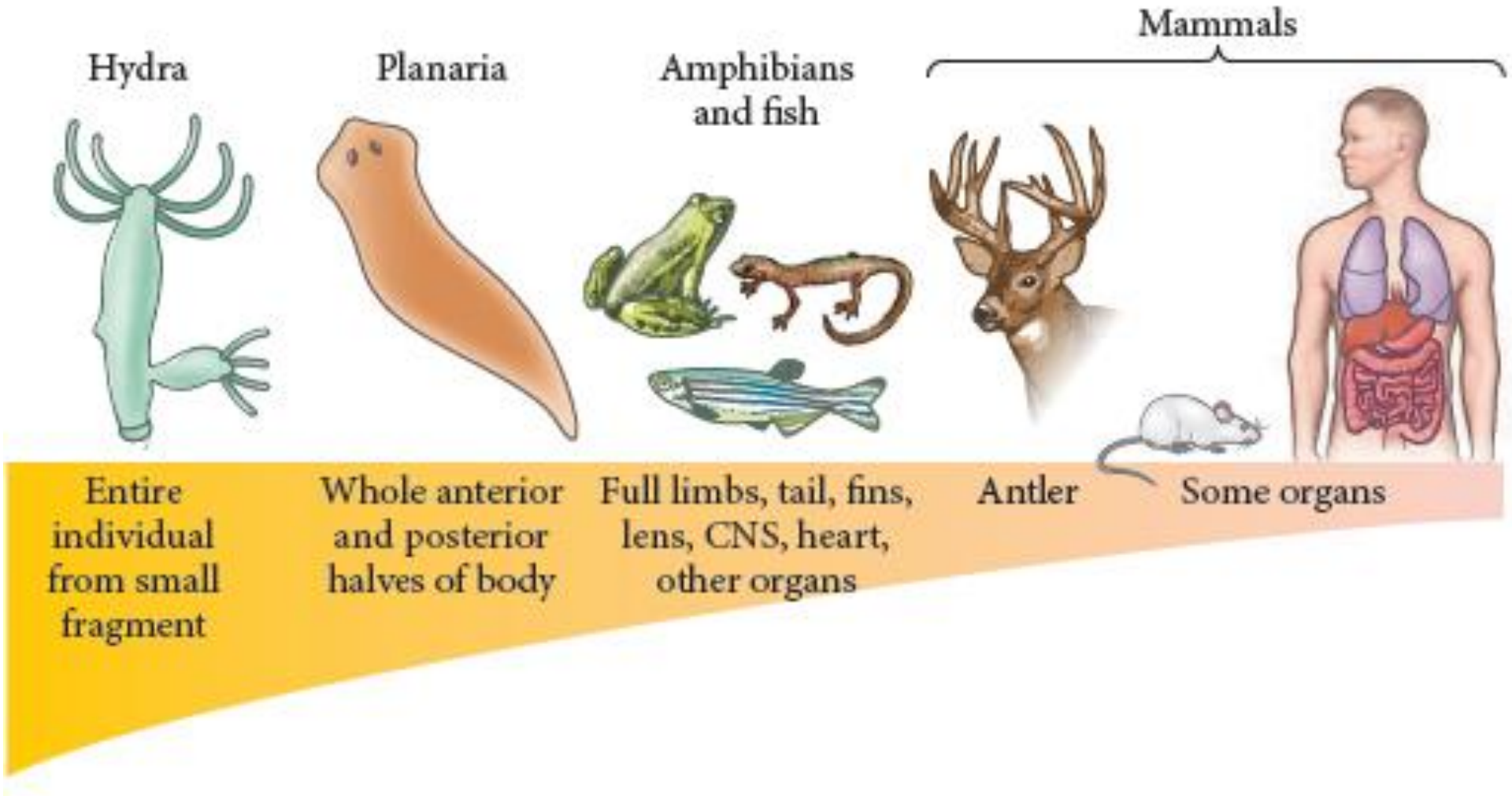






Baker et al 2007

Regeneración



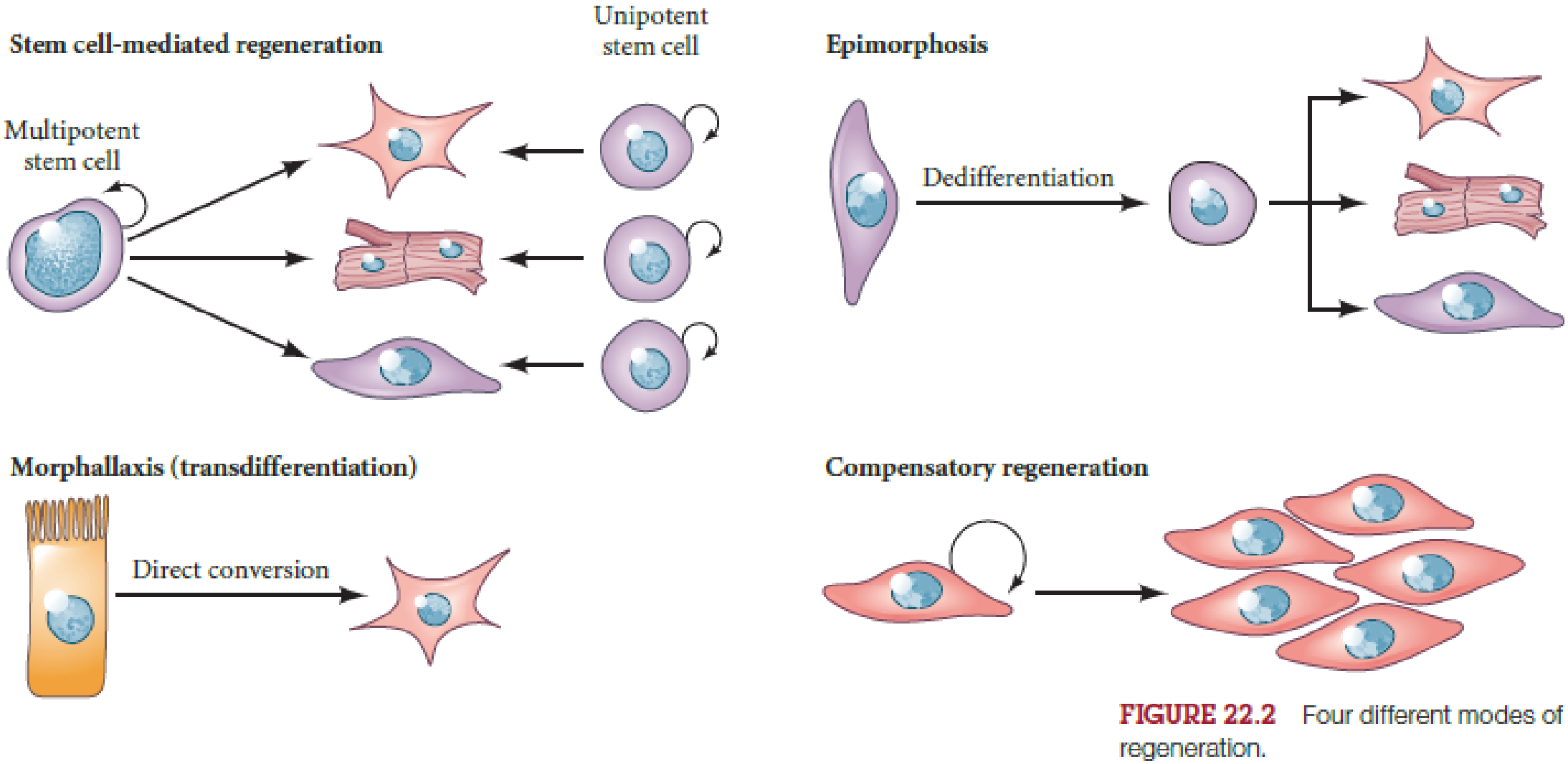


FIGURE 22.2 Four different modes of regeneration.