Jiří K a ň k a

Embryonální vývoj savců

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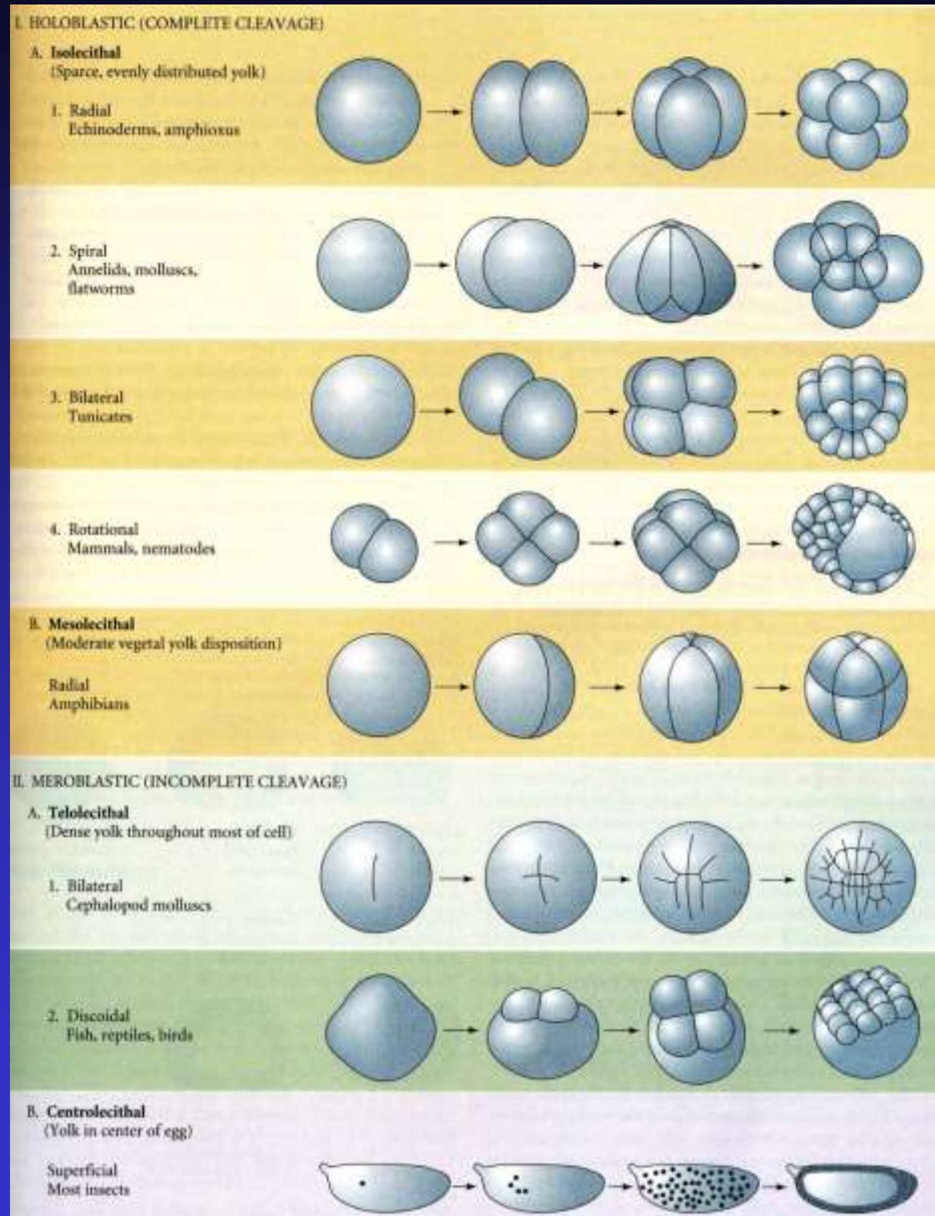
Summary of the main patterns of cleavage

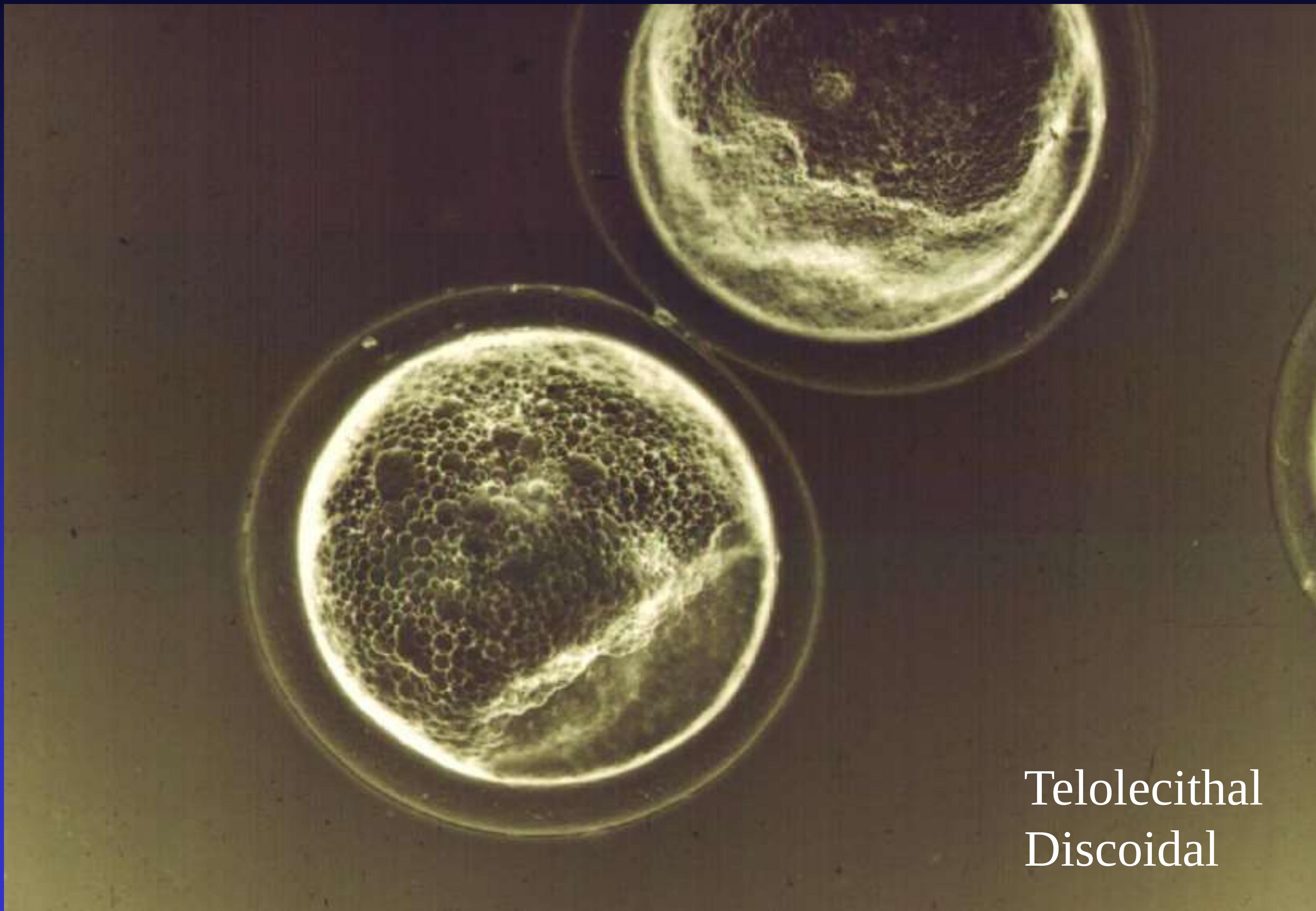
Isolecithal

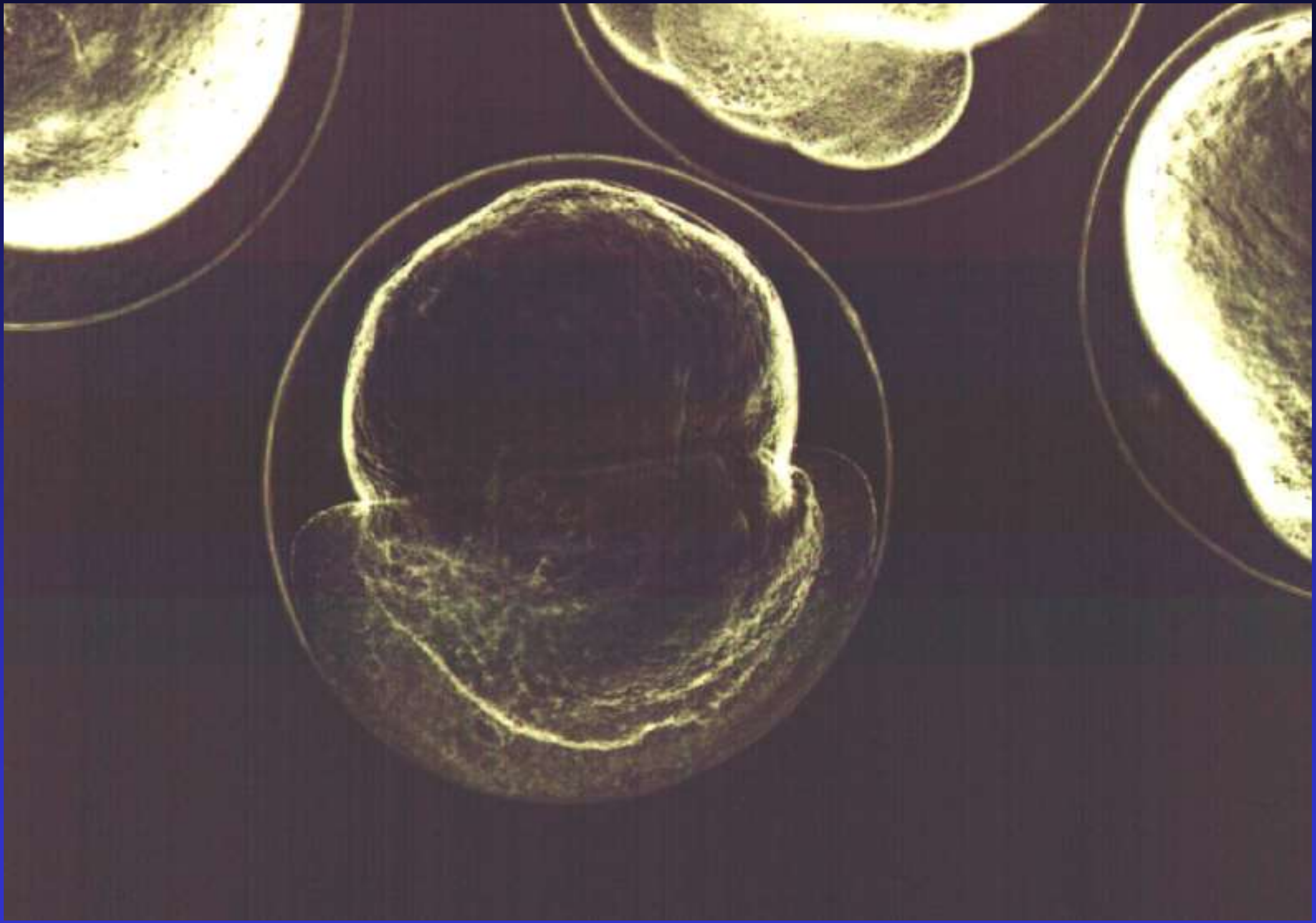
Mesolecithal

Telolecithal

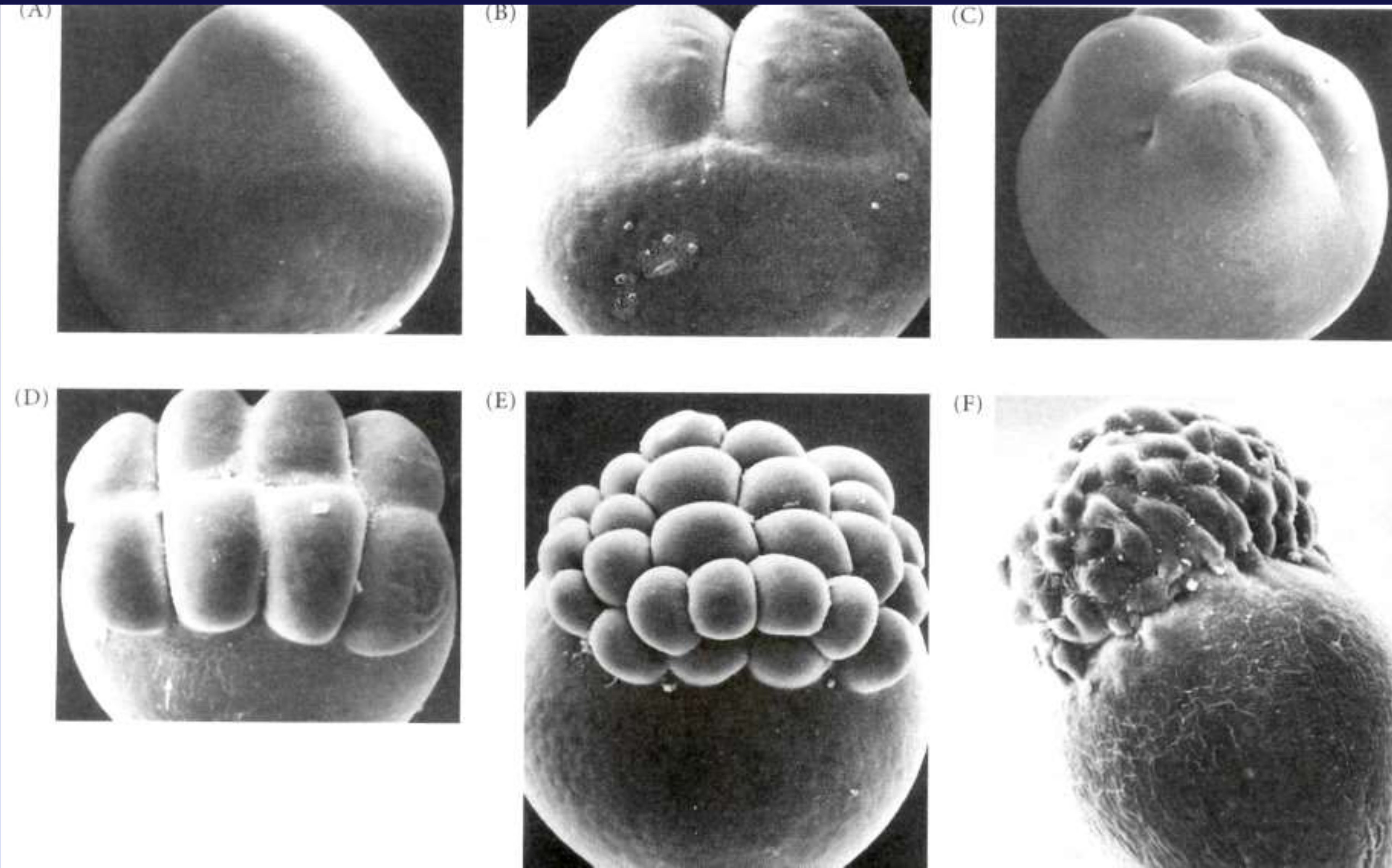
Centrolecithal

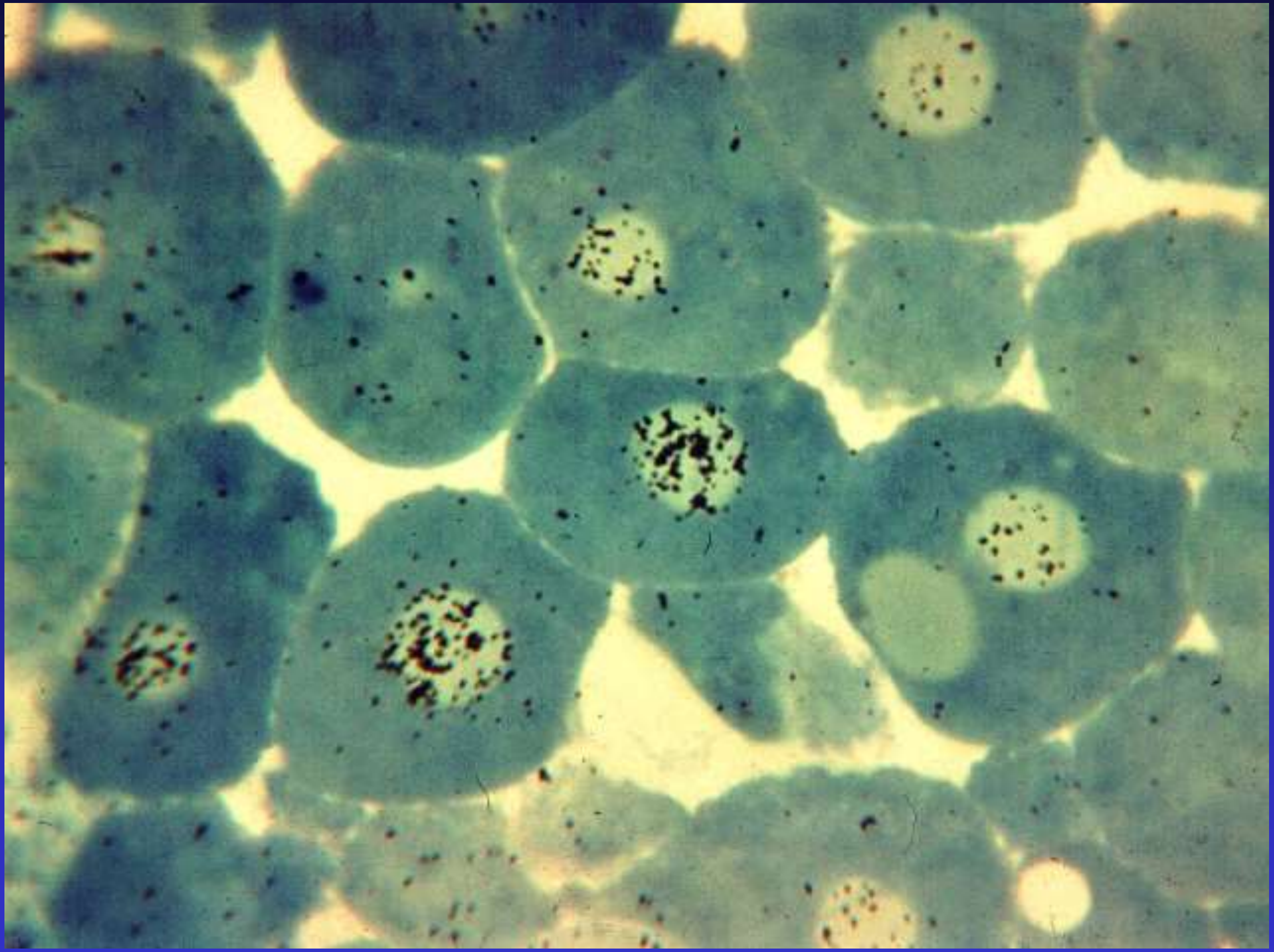


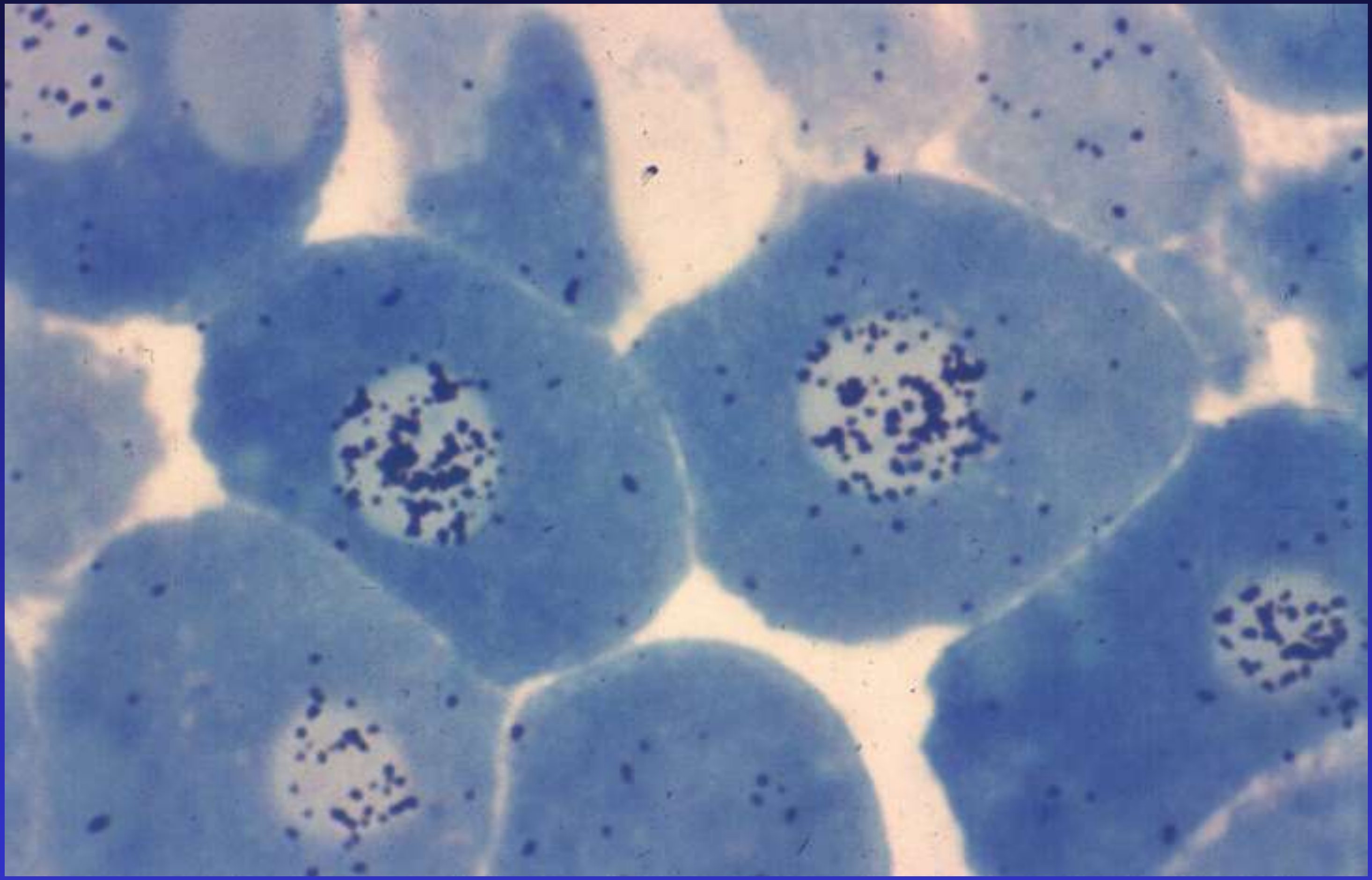

Telolecithal
Discoidal



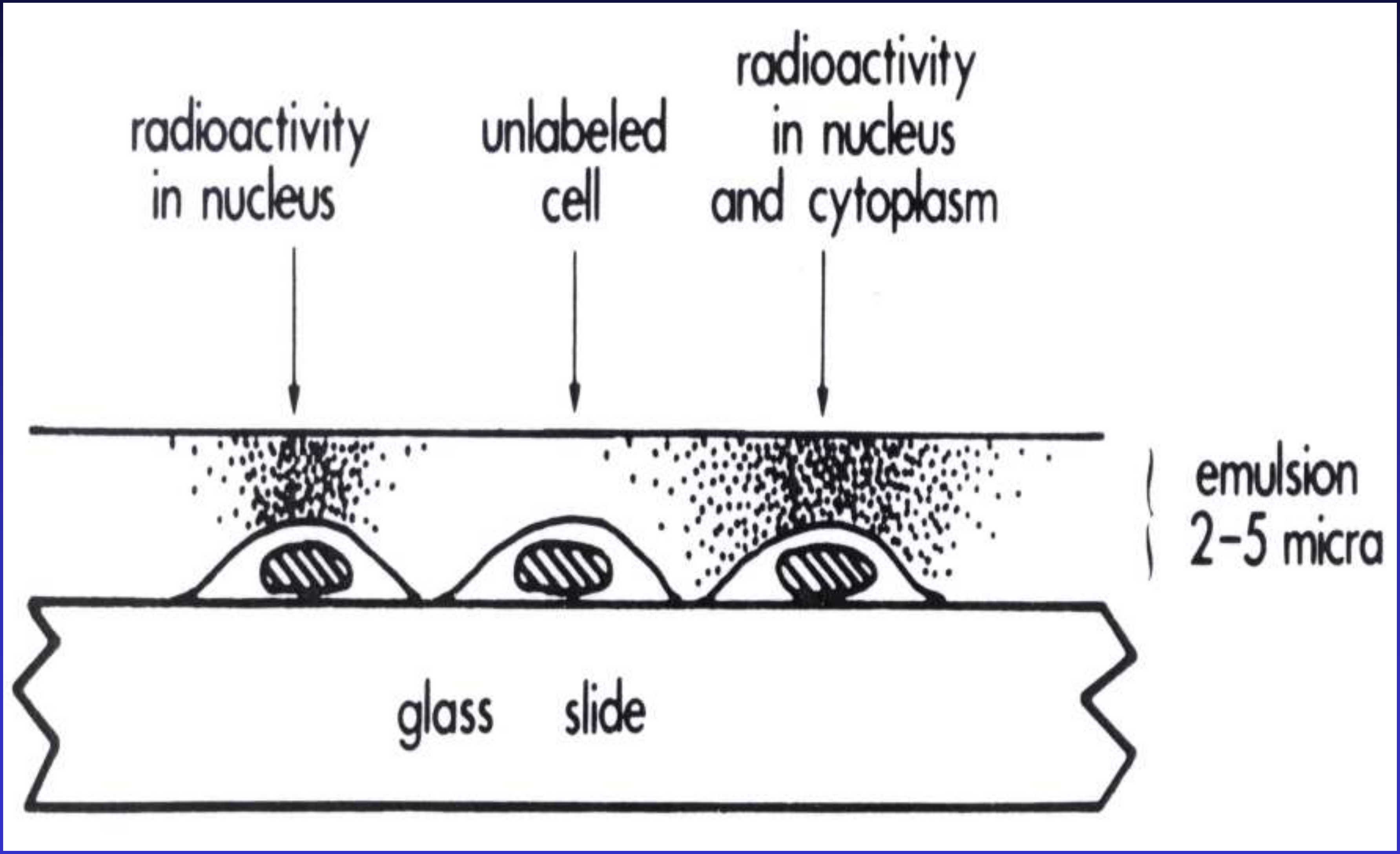
Discoidal cleavage in zebrafish egg



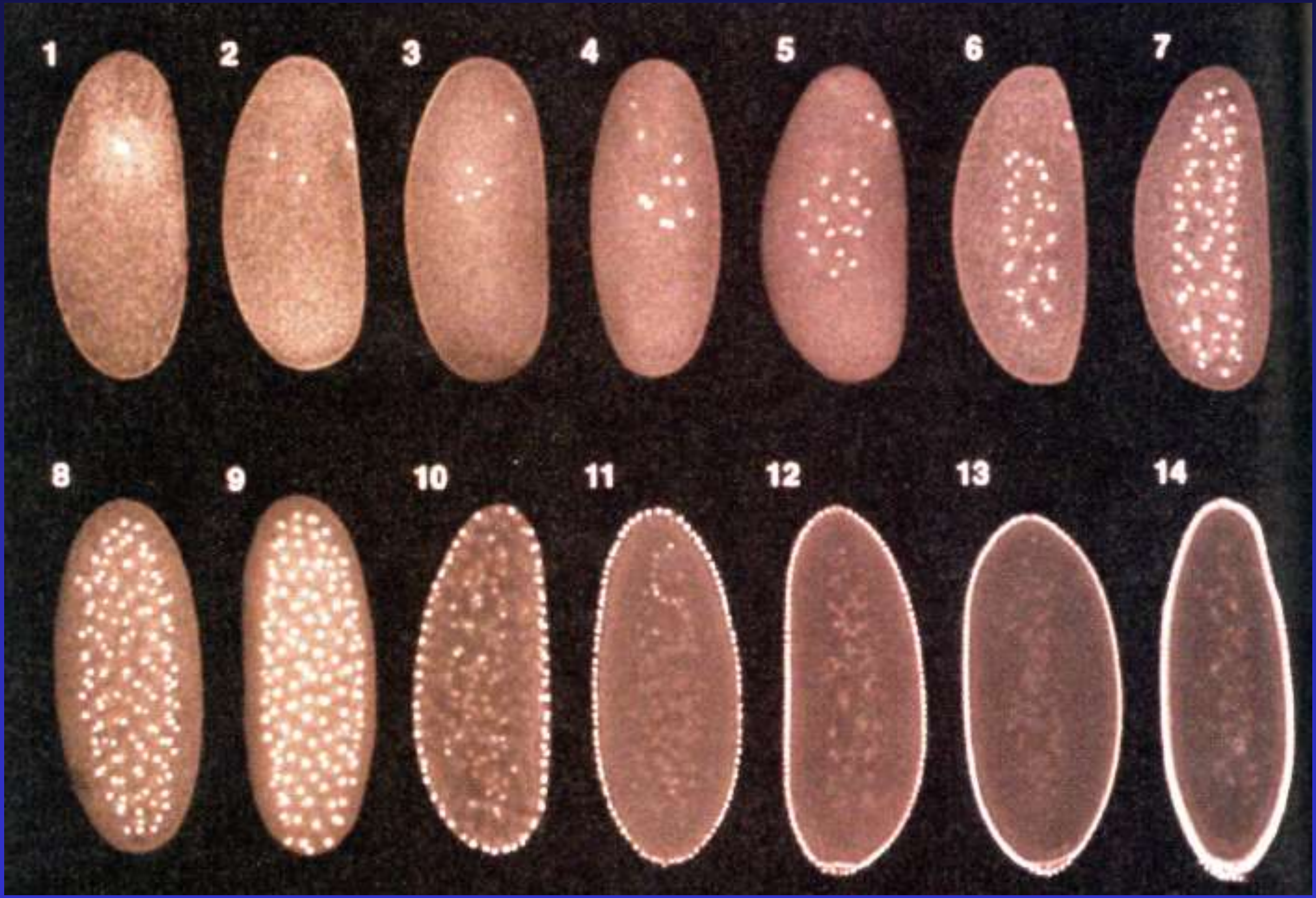




Autoradiografie

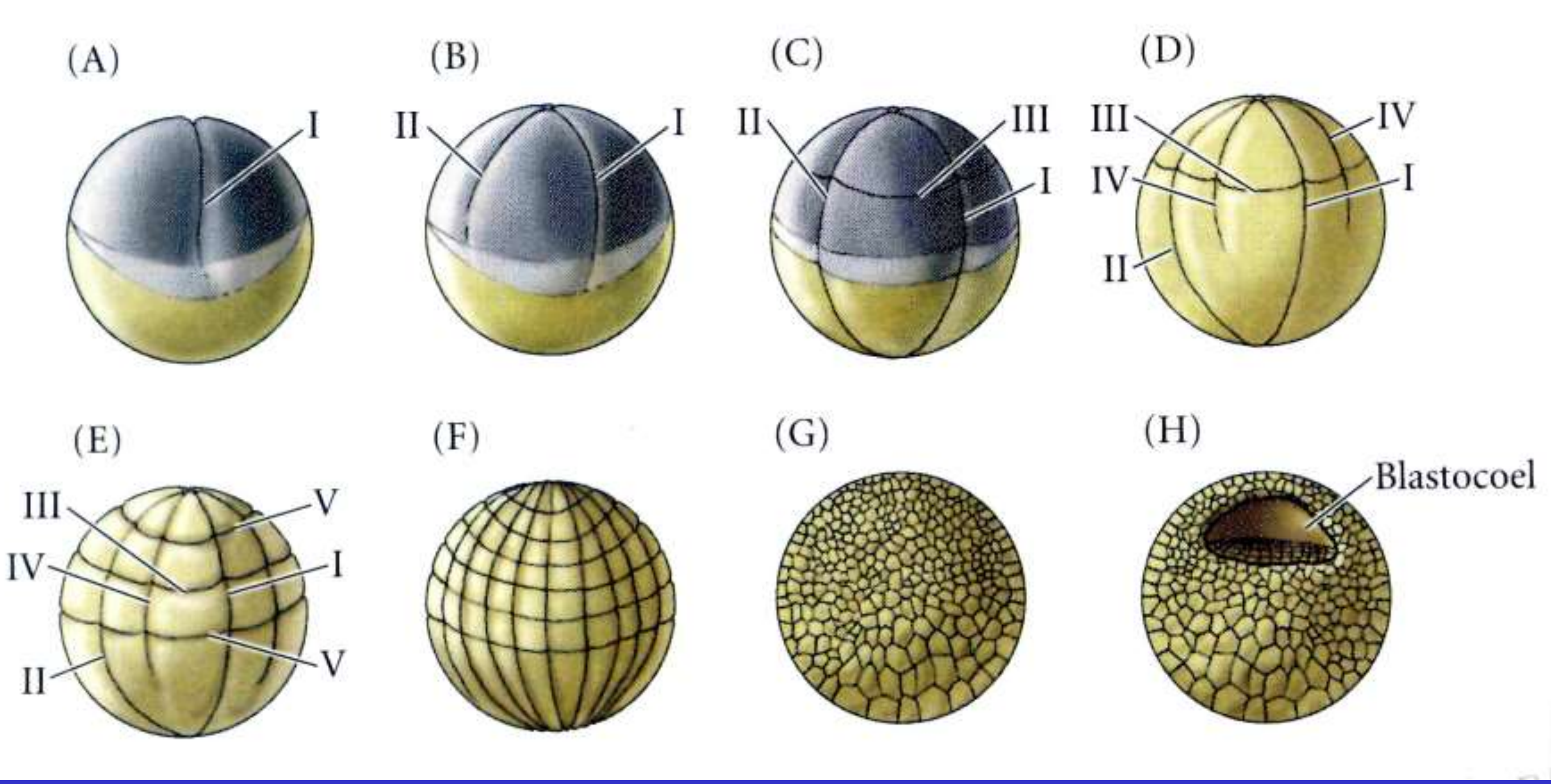


Superficial cleavage in a Drosophila embryo

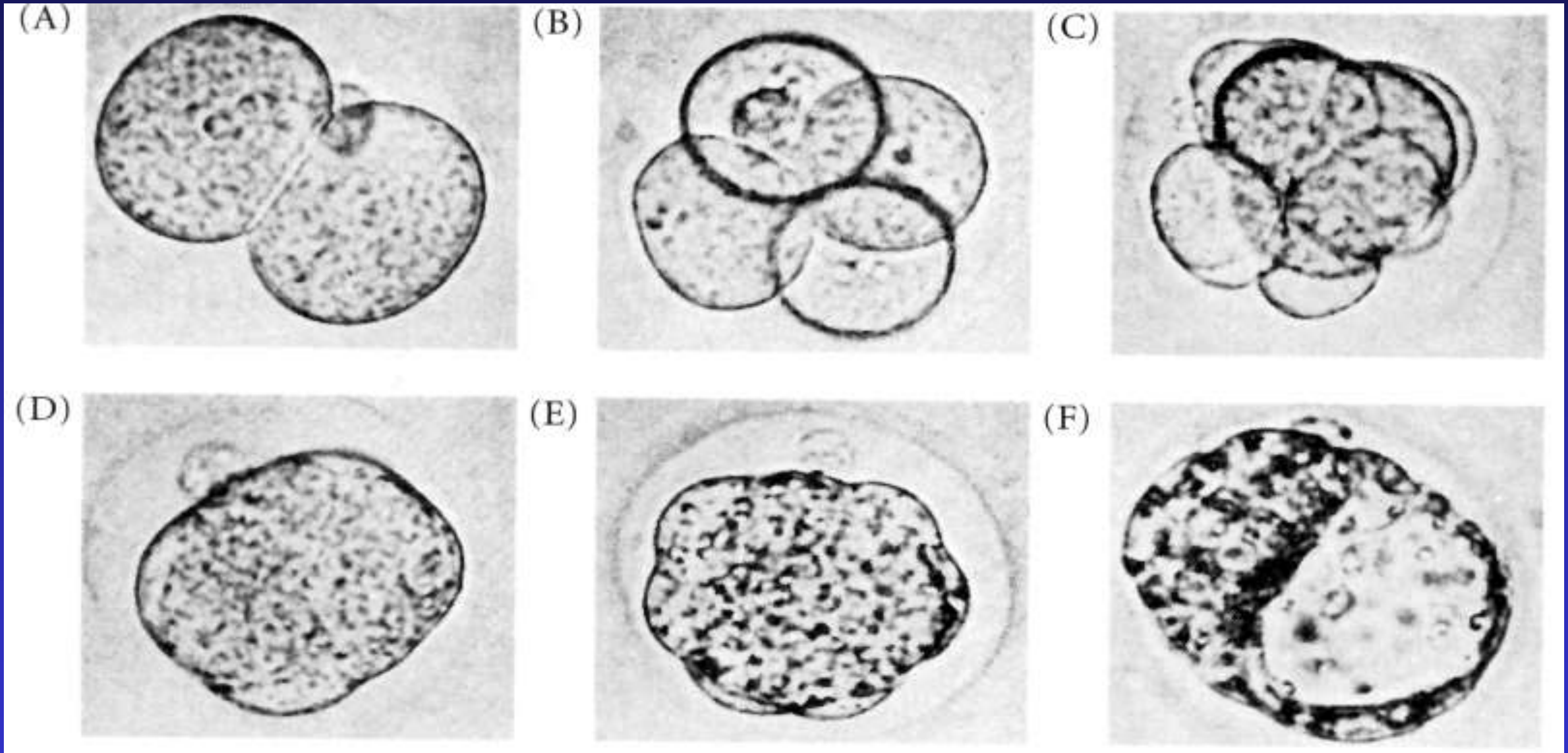


Centrolecithal

Cleavage of a frog egg (Mesolecithal, radial)



Cleavage of a single mouse in vitro



Isolecithal

Mus musculus

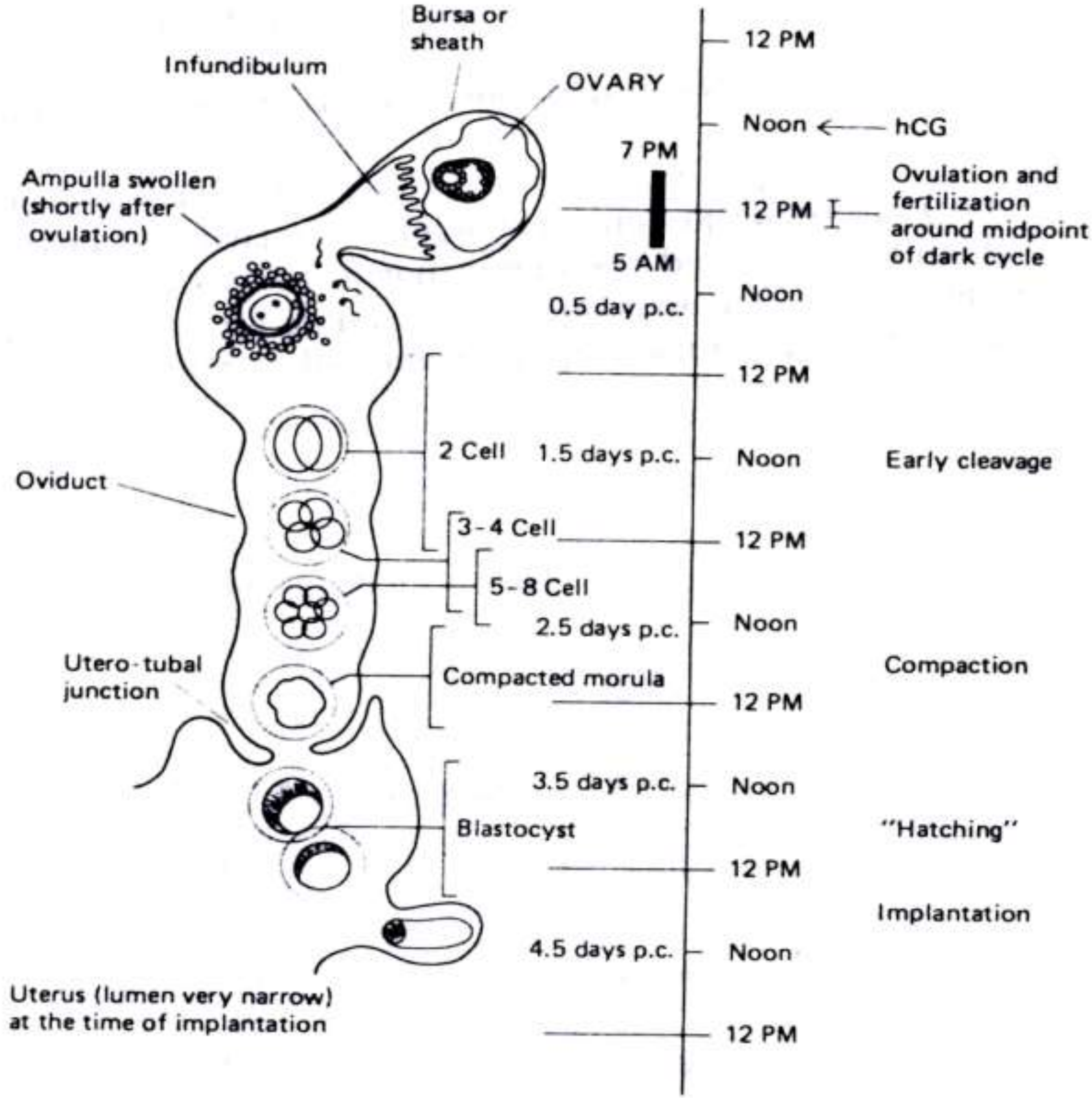
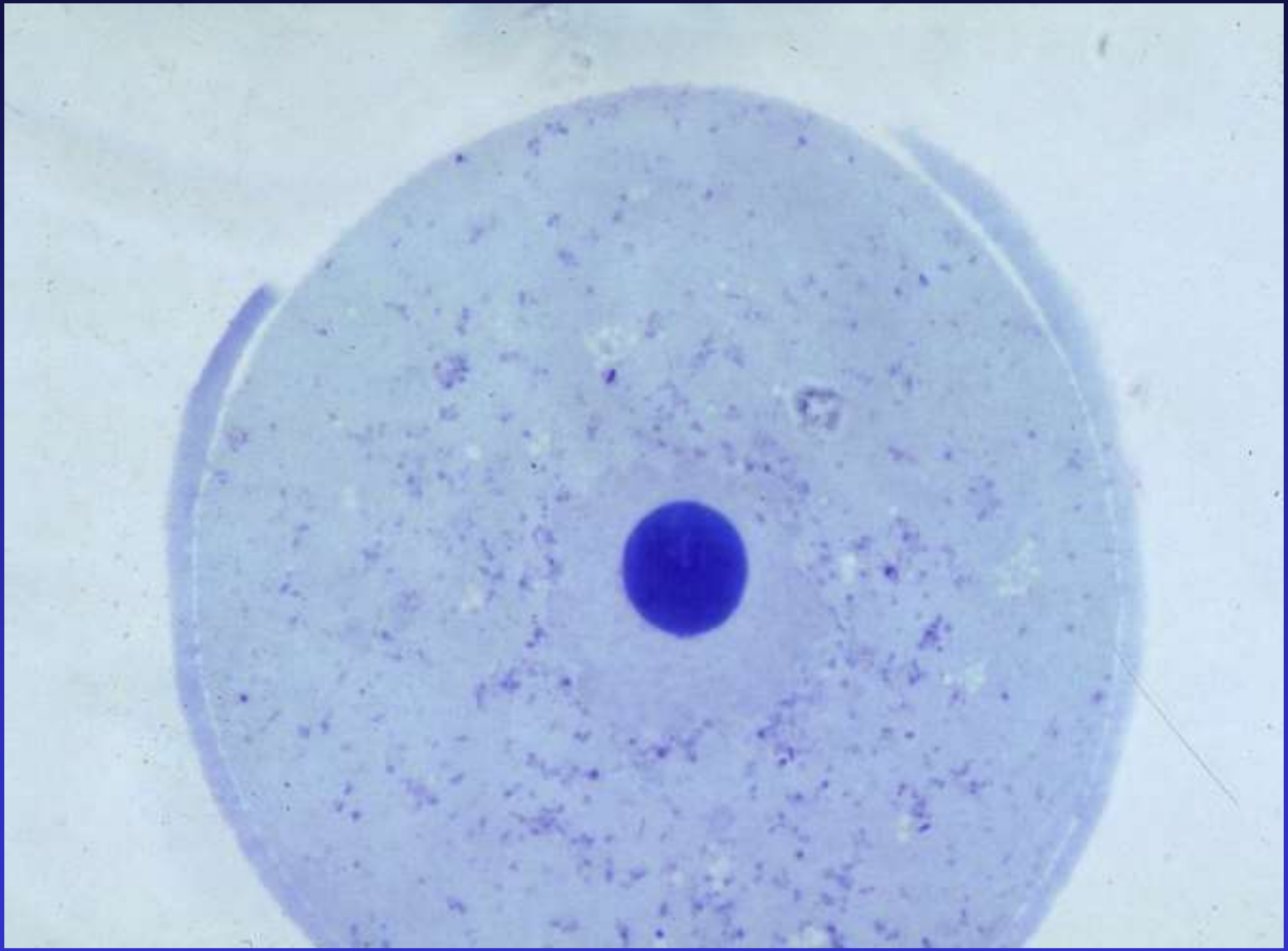
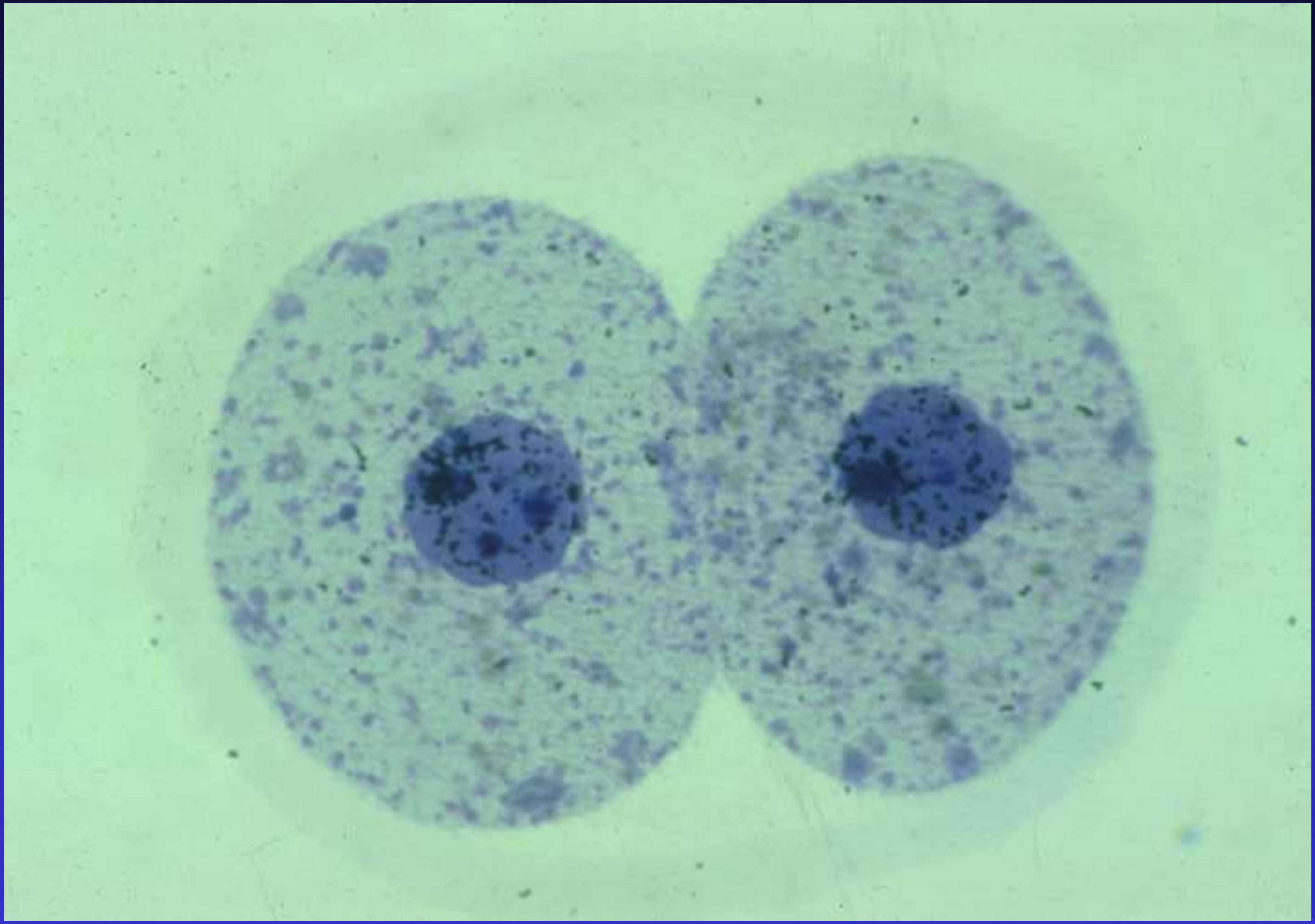
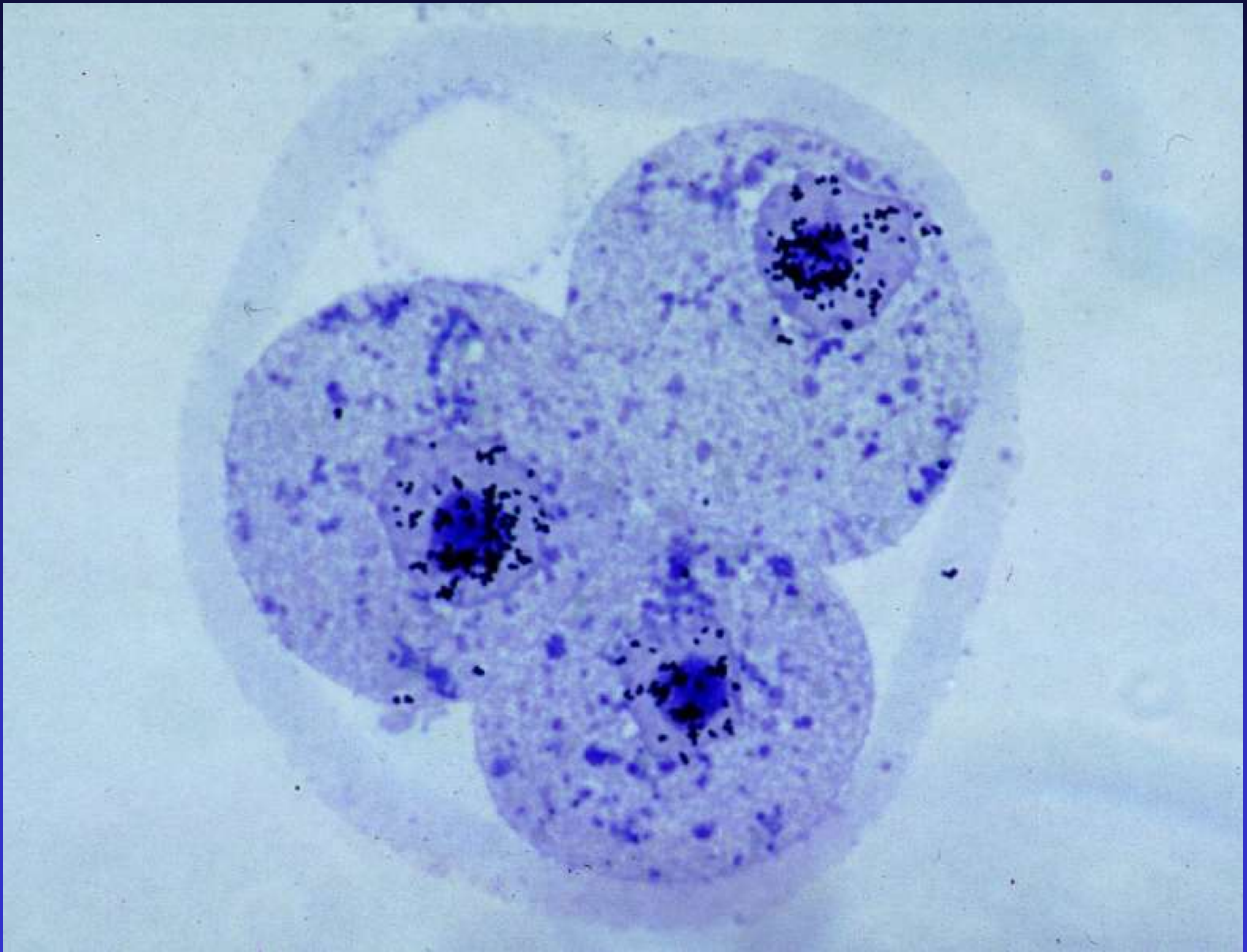


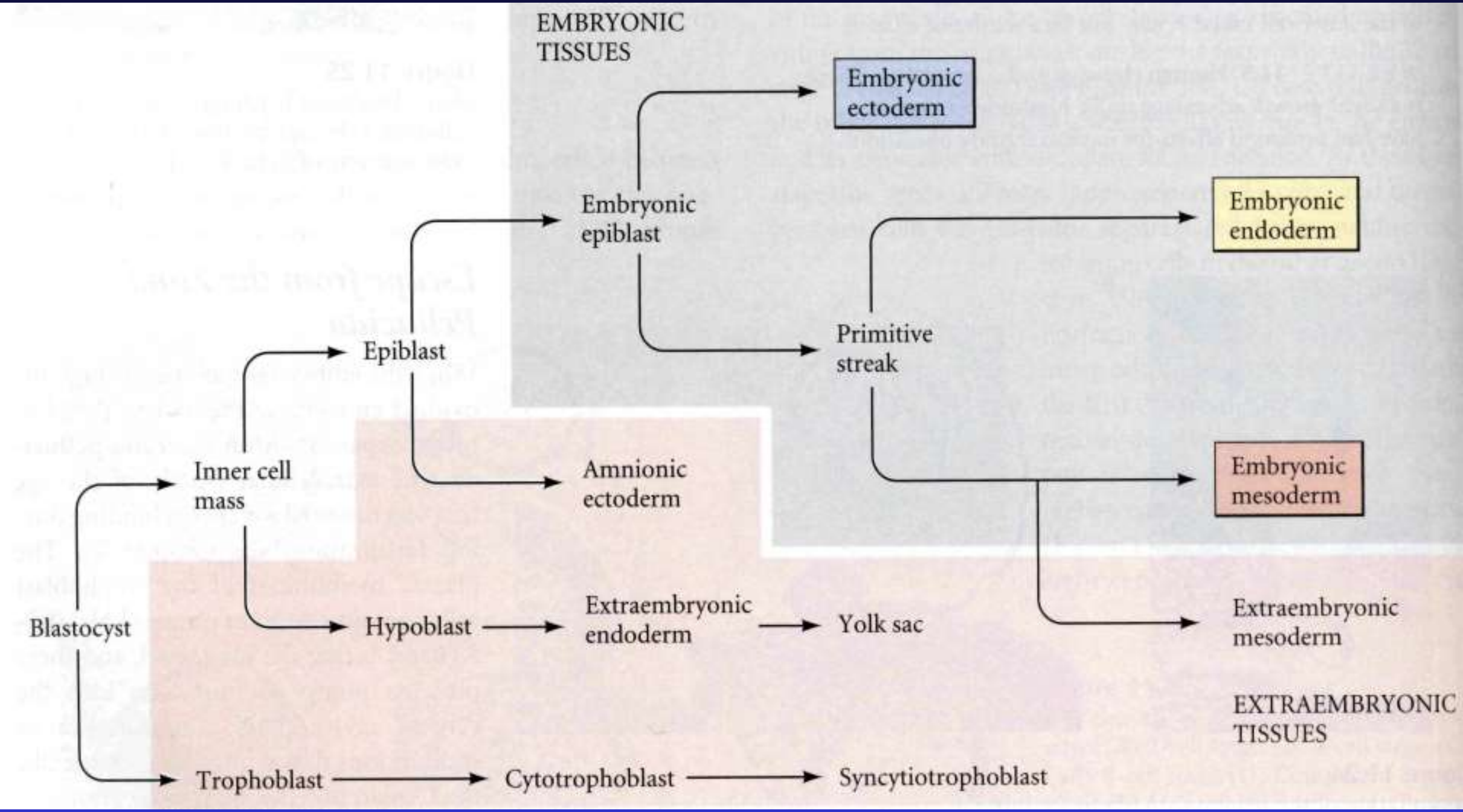
Figure 9 Summary of preimplantation development.







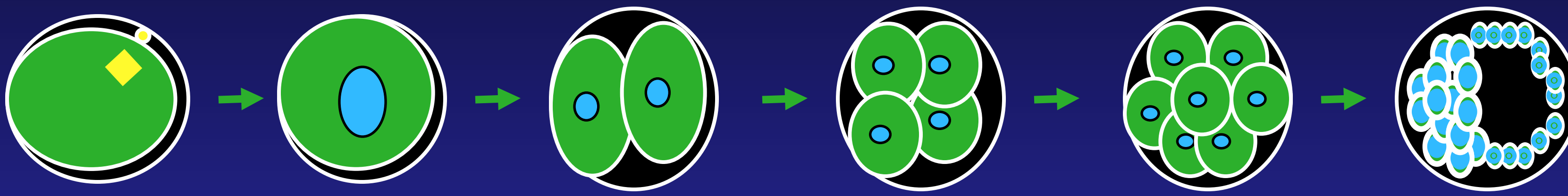
Derivation of tissues in human and rhesus monkey embryos



Gene expression

Embryonic genome activation (EGA)

mouse



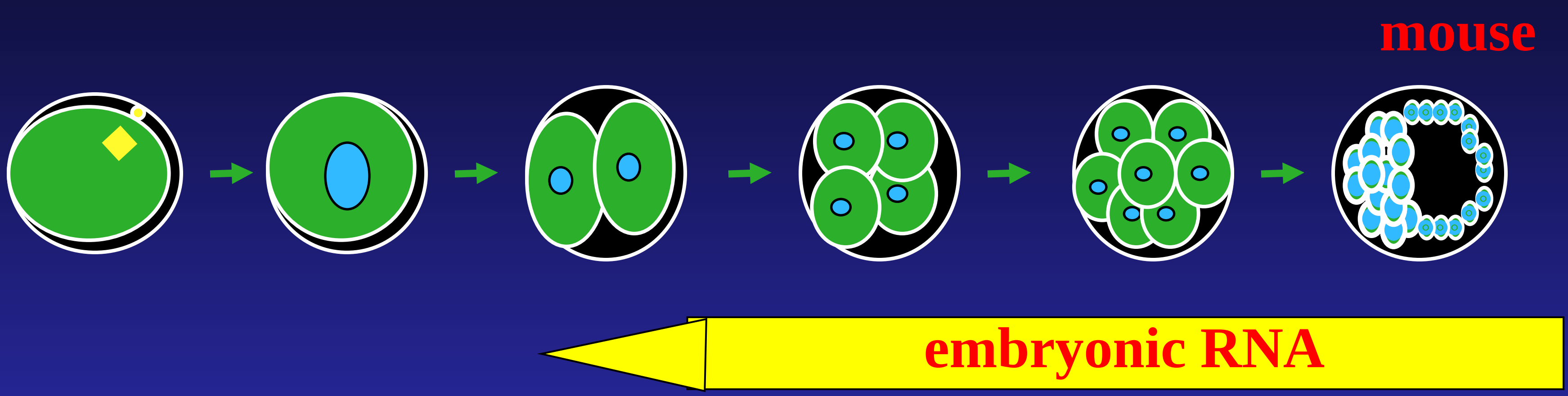
Maternal RNA

embryonic RNA

Pre-implantation period of mammalian development is characterized by important developmental transitions that occur following fertilisation

In vitro **culture system**
cryopreservation techniques
nuclear transfer techniques

Development of ideas about embryonic genome activation (EGA)

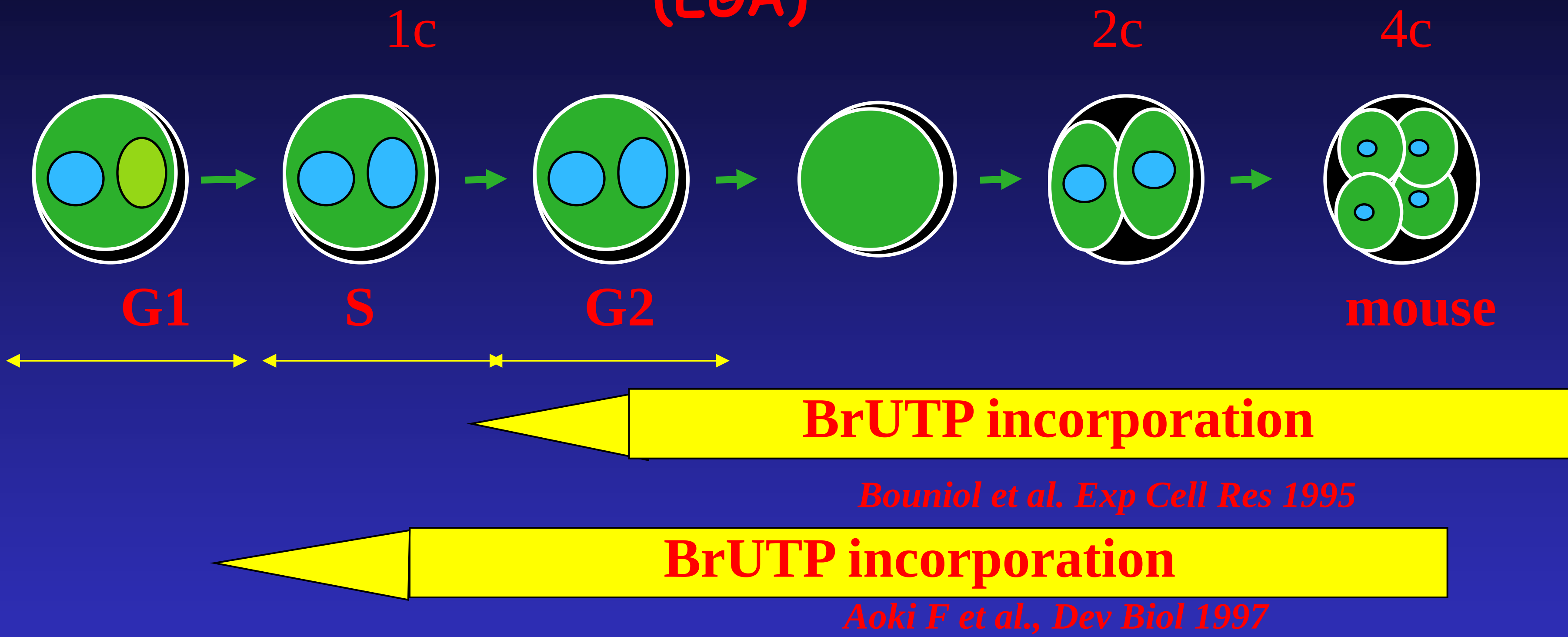


-poly (A)+ and poly (A)- RNA synthesis revealed transcription at the 2-cell stage (*Clegg, Piko, Nature 1982; J Embryol Exp Morph 1983*).

-HSP 68 and HSP 70 (*Bensaude et al., Nature 1983*), 67kDa polypeptides (*Flach et al., EMBO J 1982*), which synthesis it was possible to inhibit by α-amanitin

- EGA starts at the 2-cell stage, with a minor activation event at the early 2-cell stage and major activation event at the late 2-cell stage.

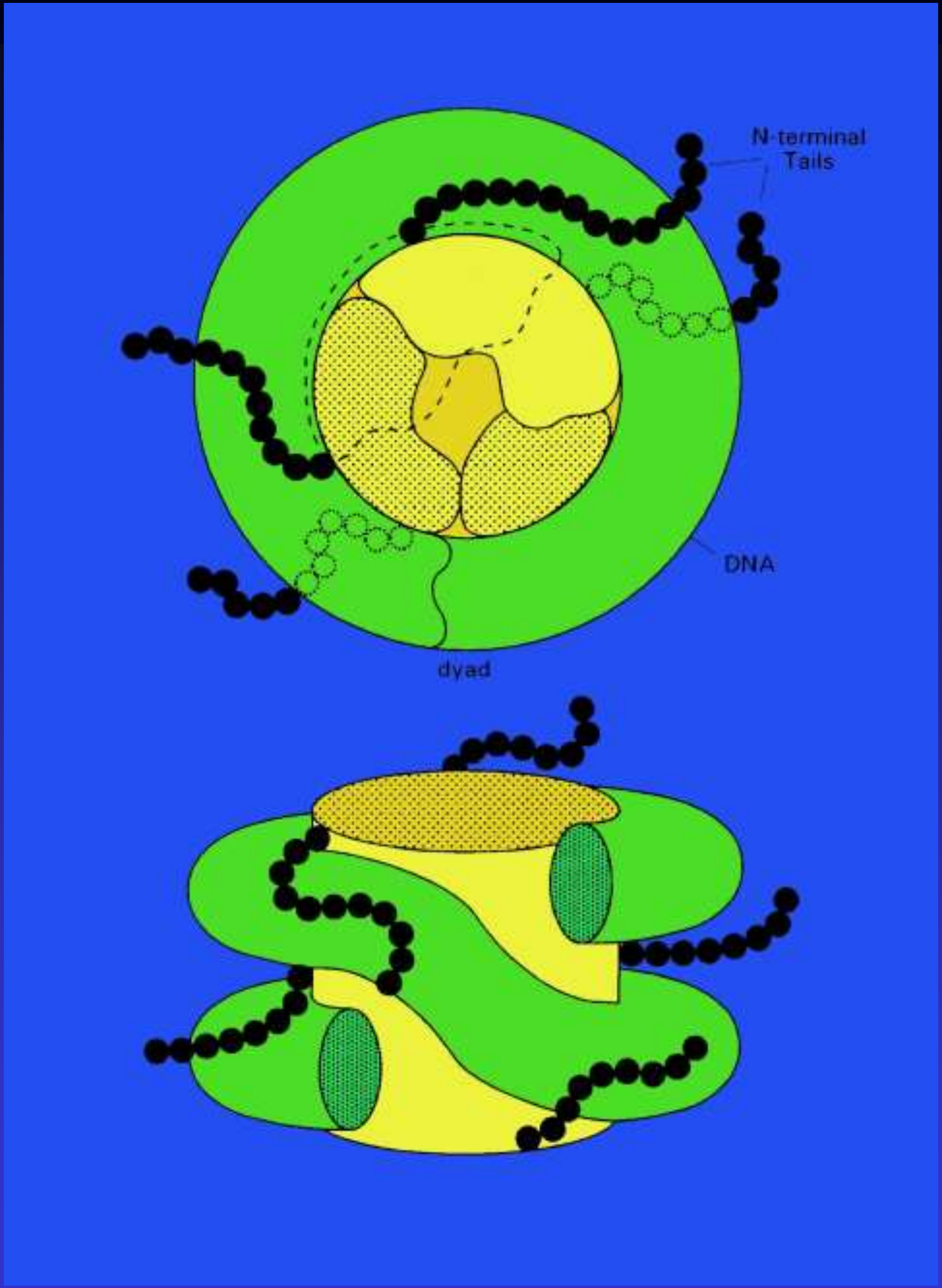
Development of ideas about embryonic genome activation (EGA)



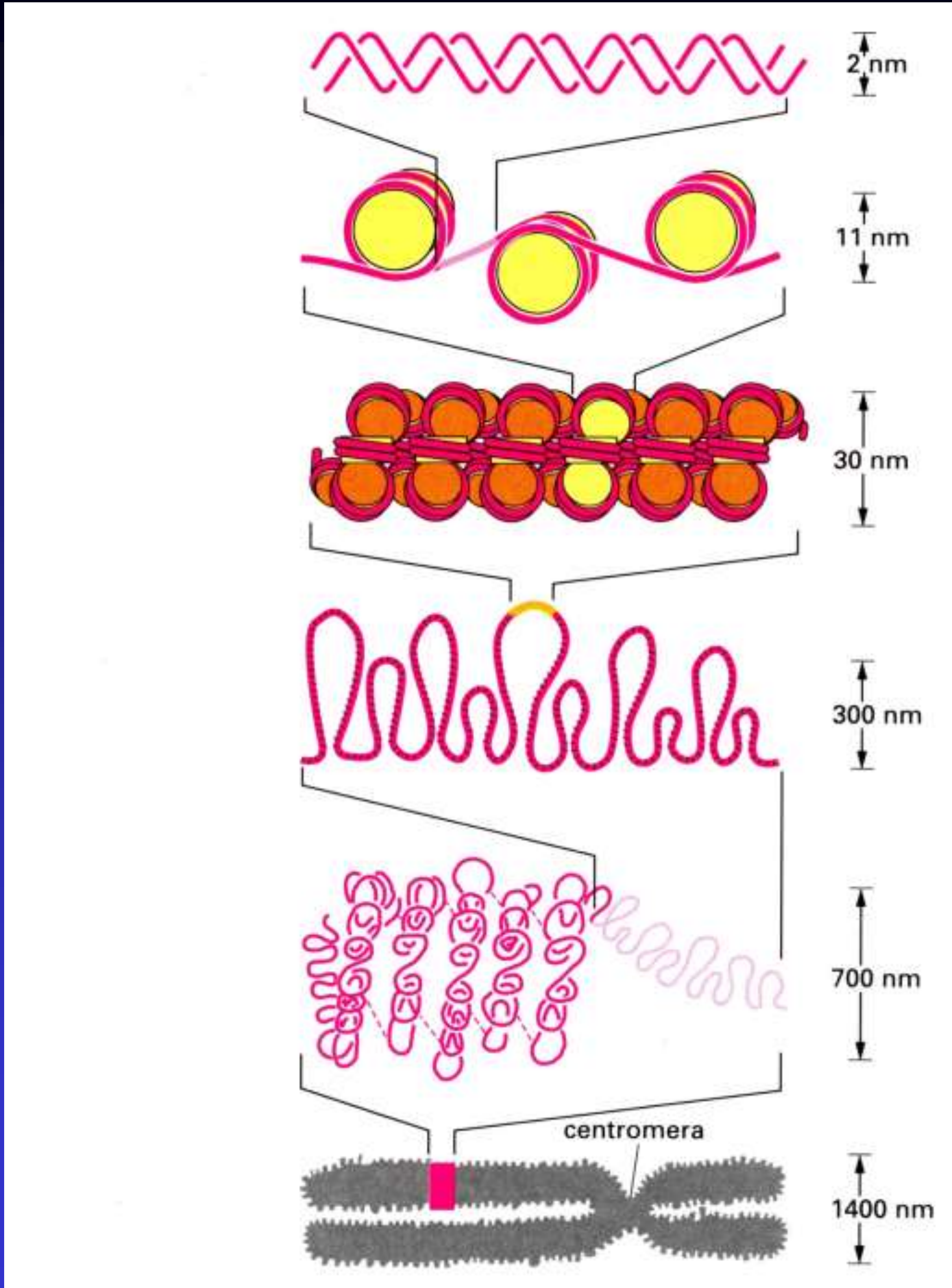
Late 1-cell stage supports the transcription of some 2-cell stage specific genes within transplanted 2-cell stage nuclei (U2afbp-rs, ERV-L, 70 kDa TRC complex) ***(Latham, Schultz Frontiers in Bioscience, 2001)***

These studies support the hypothesis that genome activation occurs in a stepwise manner in the mouse embryo

Chromatin structure



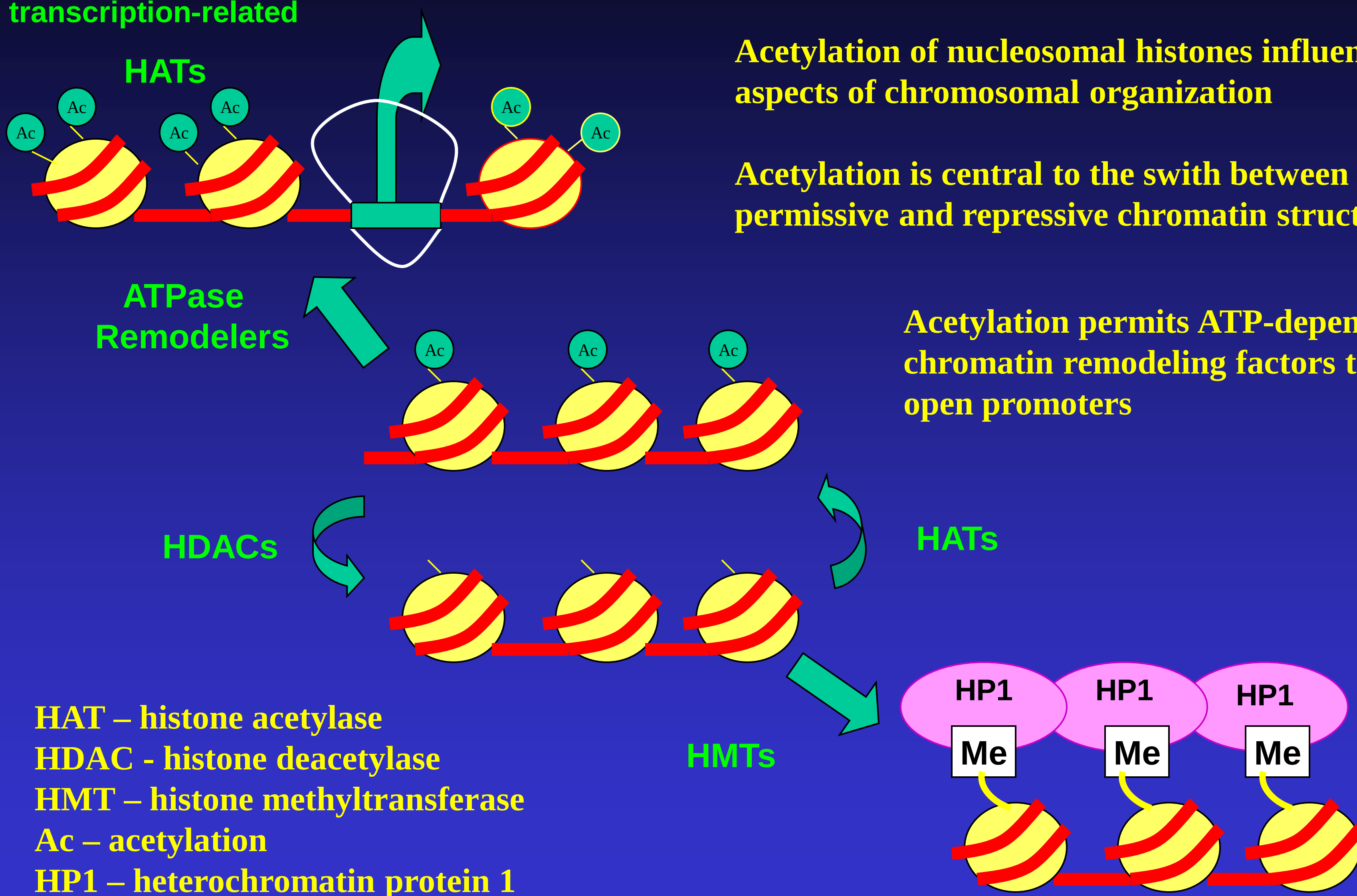
Two molecules of each of the four core histones (H2A, H2B, H3 and H4) wrap 160 bp of DNA. N-termini (19 and 26 amino acids) of the core histones H3 and H4 are among the most highly conserved sequences in eukaryotes.



Chromatin structure

single molecule of linker histone (H1) organize 180–190 bp of DNA

Histone acetylation



Acetylation of nucleosomal histones influences all aspects of chromosomal organization

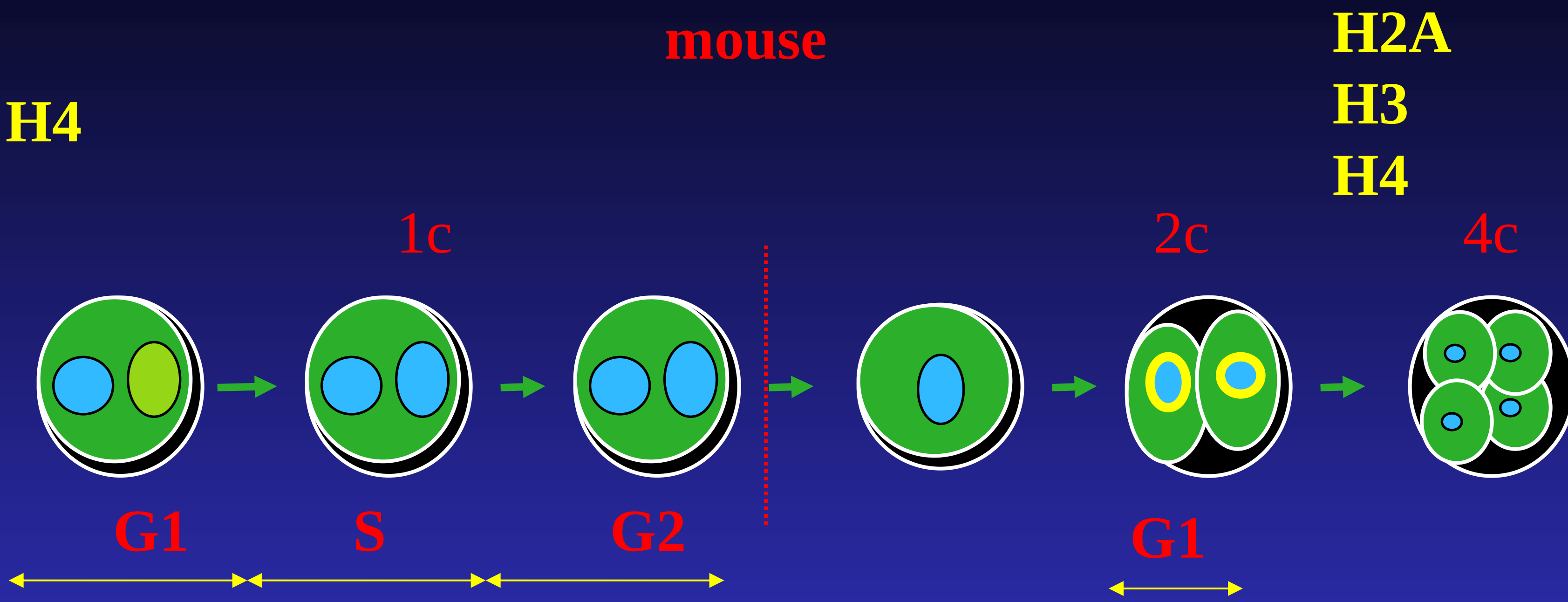
Acetylation is central to the swith between permissive and repressive chromatin structure

Acetylation permits ATP-dependent chromatin remodeling factors to open promoters

HAT – histone acetylase
HDAC - histone deacetylase
HMT – histone methyltransferase
Ac – acetylation
HP1 – heterochromatin protein 1

Histone acetylation

mouse



hyperacetylated H4 - G_1 phase, the male pronuclei exibited higher levels

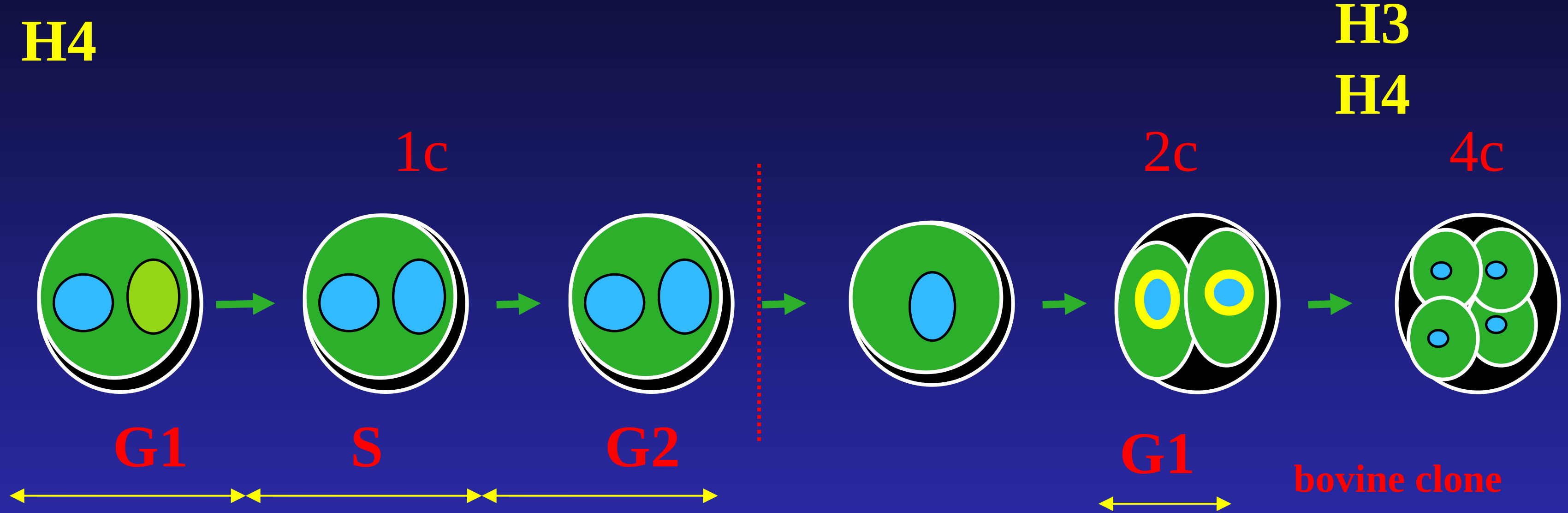
Worrad et al., Development 1995

Stein et al., Mol Reprod Dev 1997

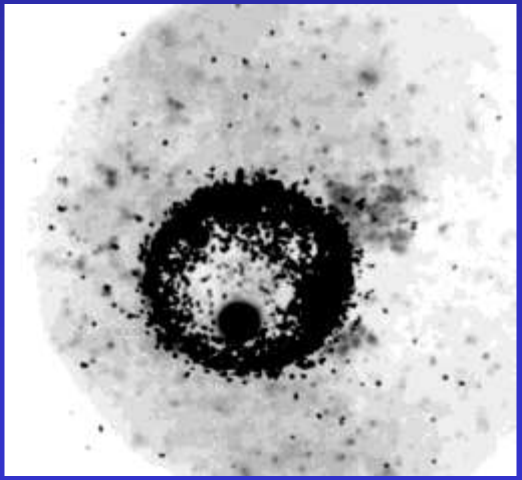
H4 acetylated on lysine 16 is uniformly distributed in the nucleoplasm of 1- and 2-cell stage
H4, acetylated on lysines 5, 8 and 12, is enriched at the nuclear periphery in 2-cell stage
H3 acetylated at lysine 9 and/or 18 and the single acetylated forms of H2A become transiently enriched at the nuclear periphery in the 2-cell

Histone acetylation

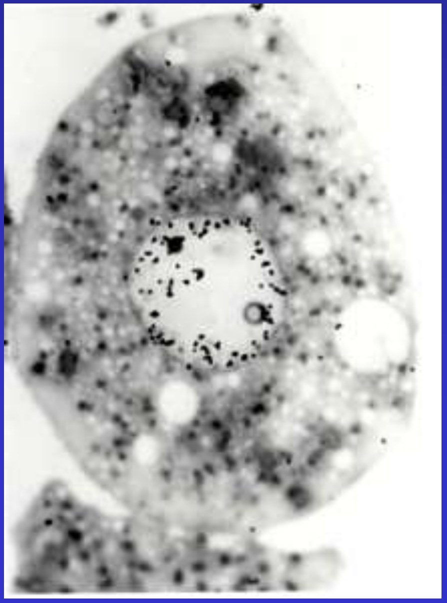
mouse



mouse



bovine clone



histones acetylation is involved in chromatin remodelling during development

DNA methylation

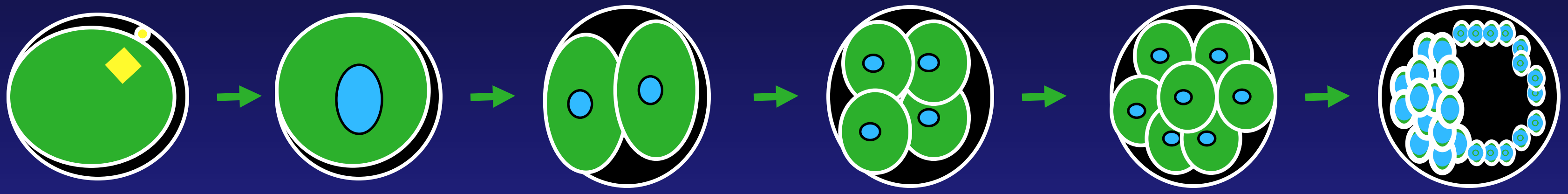
-addition of methyl groups to the 5-position of cytosine within cytosine-guanine dinucleotides (CG)

High level of methylation – gene silencing

Imprinted genes – difference of methylation status of the regulatory region determines whether the maternal or paternal derived allele is expressed

Methyltransferases – DNMT1
DNMT3A
DNMT3B

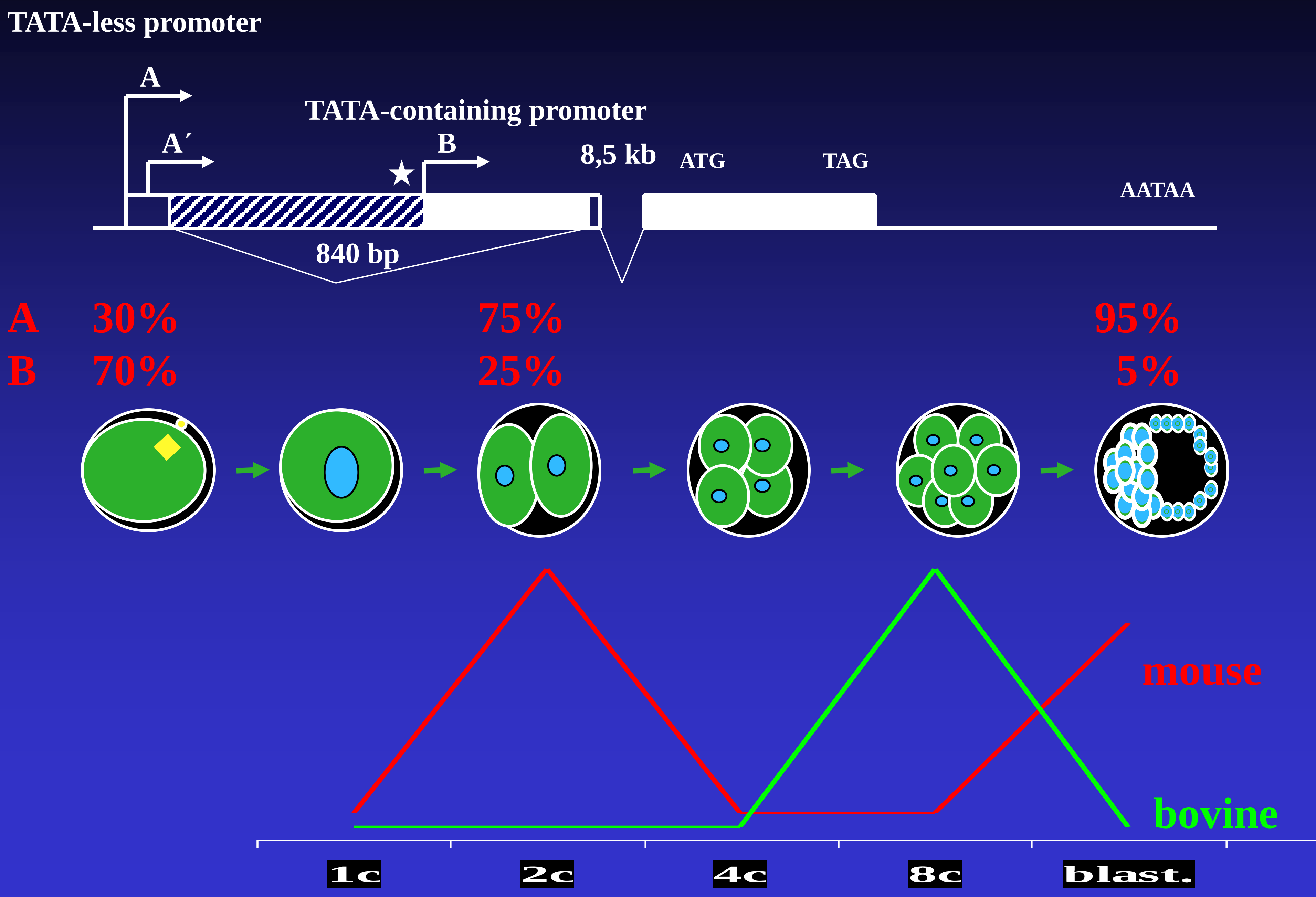
Transcription factors



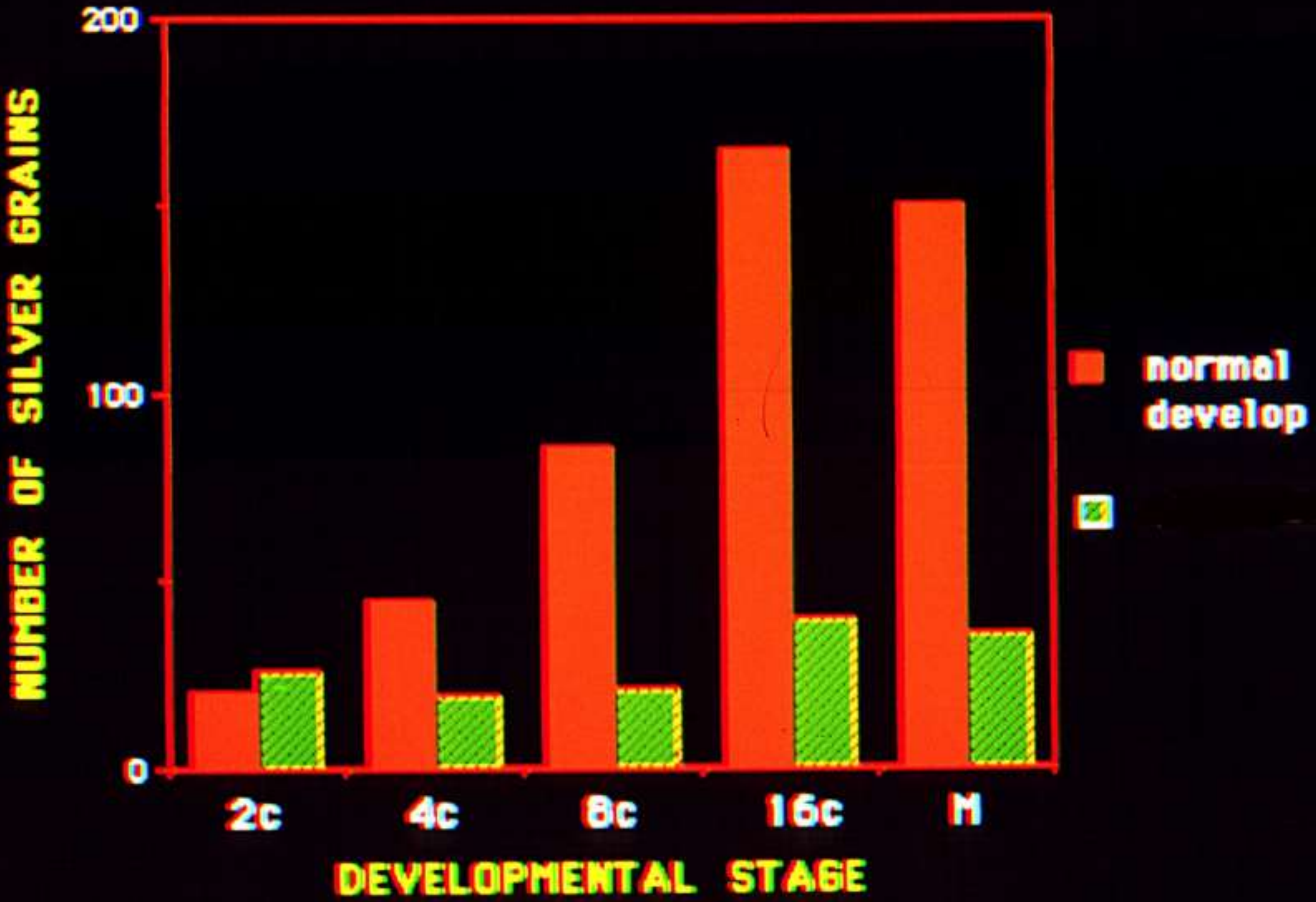
Transcriptional factors are proteins that binds to enhancer or promoter region and interact to activate or repress the transcription.

Transcriptional factors are expressed as maternally derived proteins in the early embryo. Transcript abundance of factors Sp1, TBP, CBP and mTEAD-2 starts to decrease during oocyte maturation and then increases at the 2-cell stage

Translational initiation factor eIF-1A

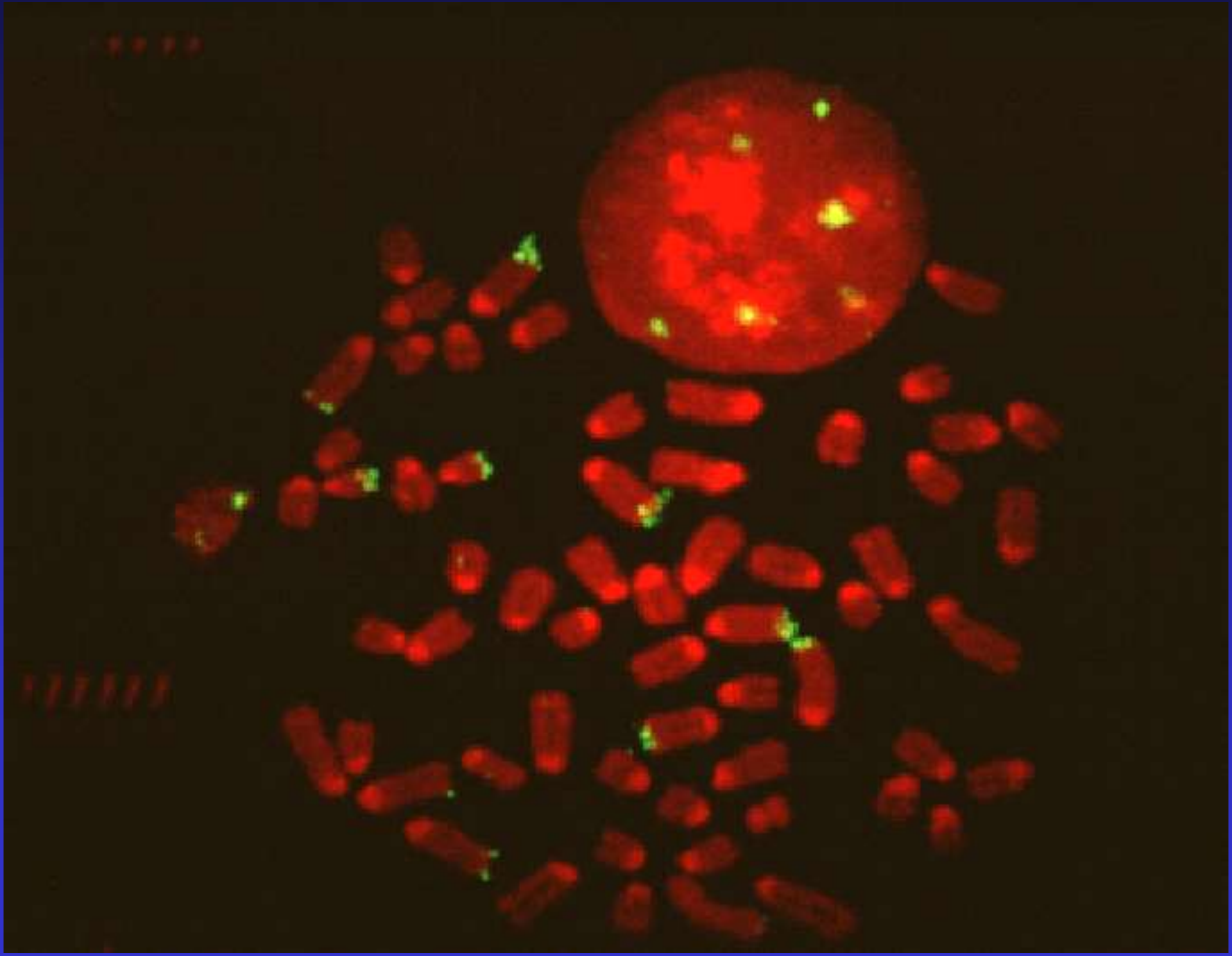


INCORPORATION OF ^3H URIDINE

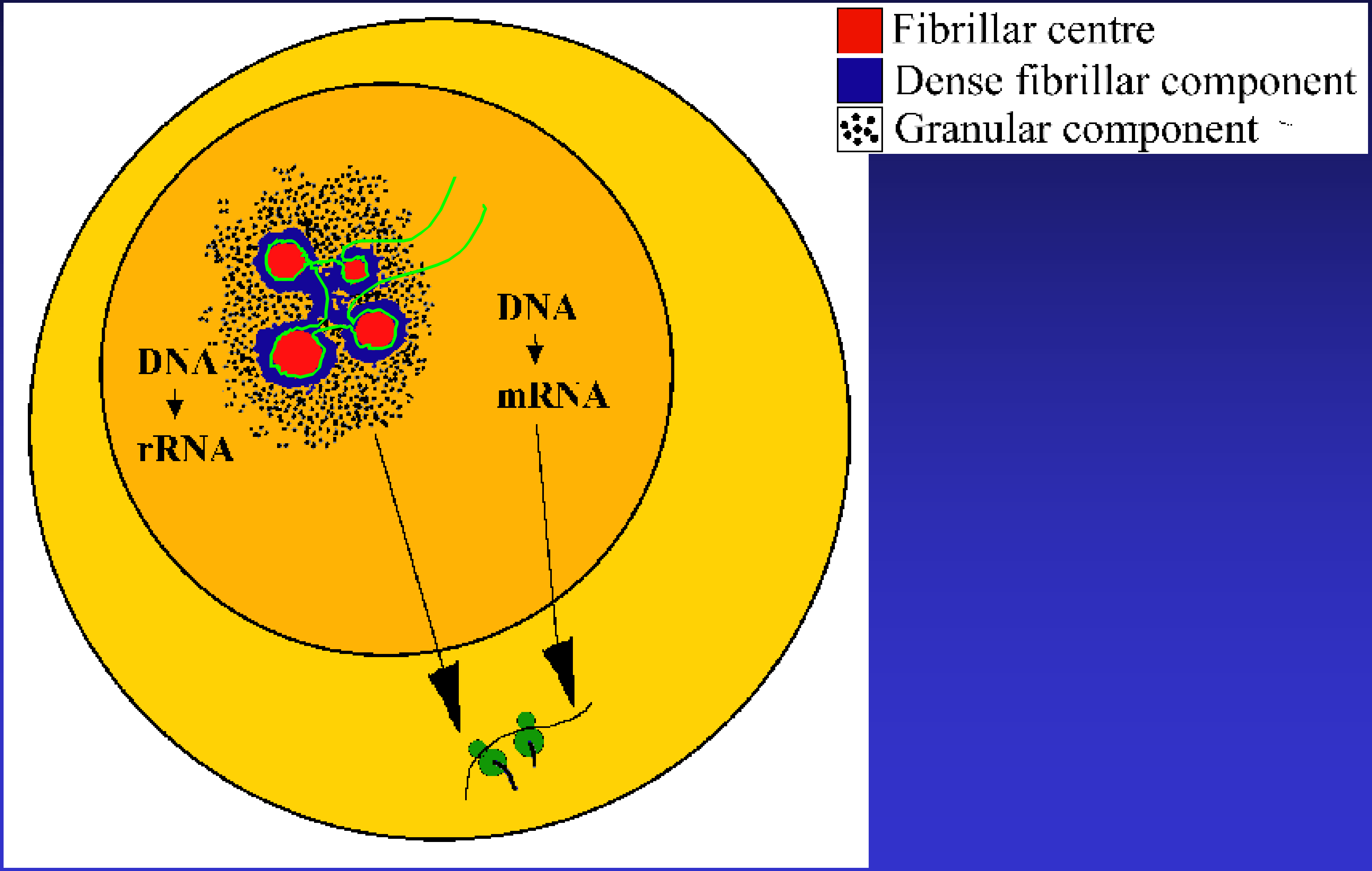


Bovine rRNA genes

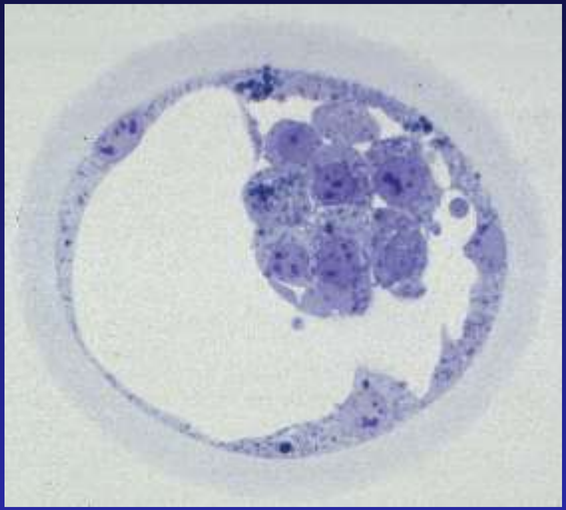
10 nucleolus organizer regions on chromosomes 2, 3, 4, 11, 28.

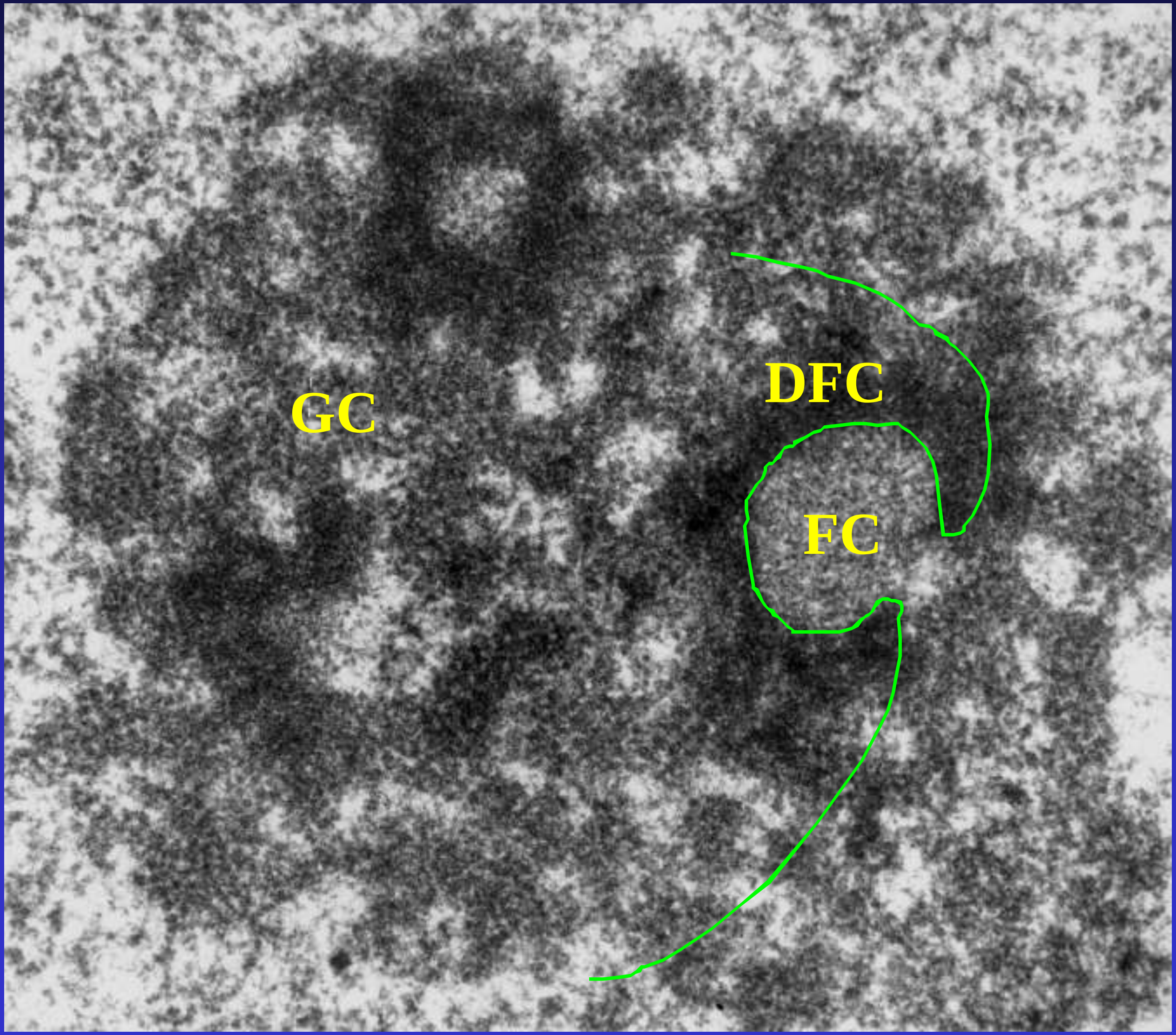


Ribosomal RNA (rRNA) genes and nucleolus

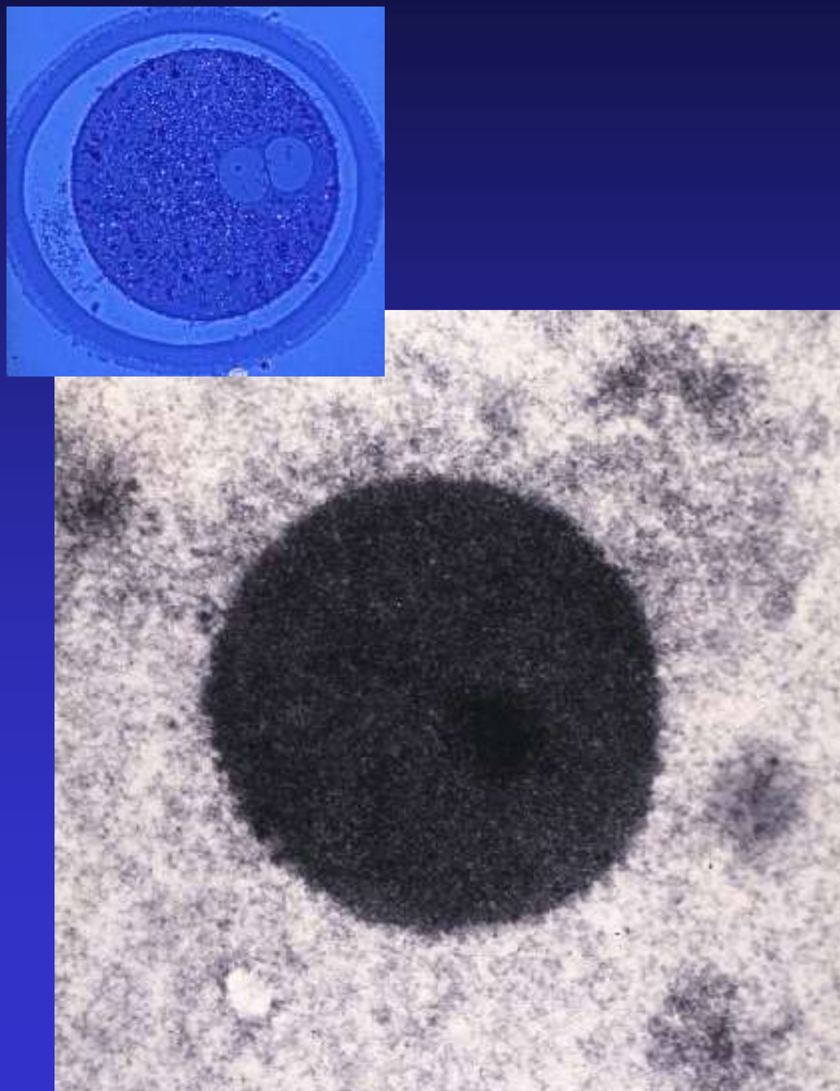


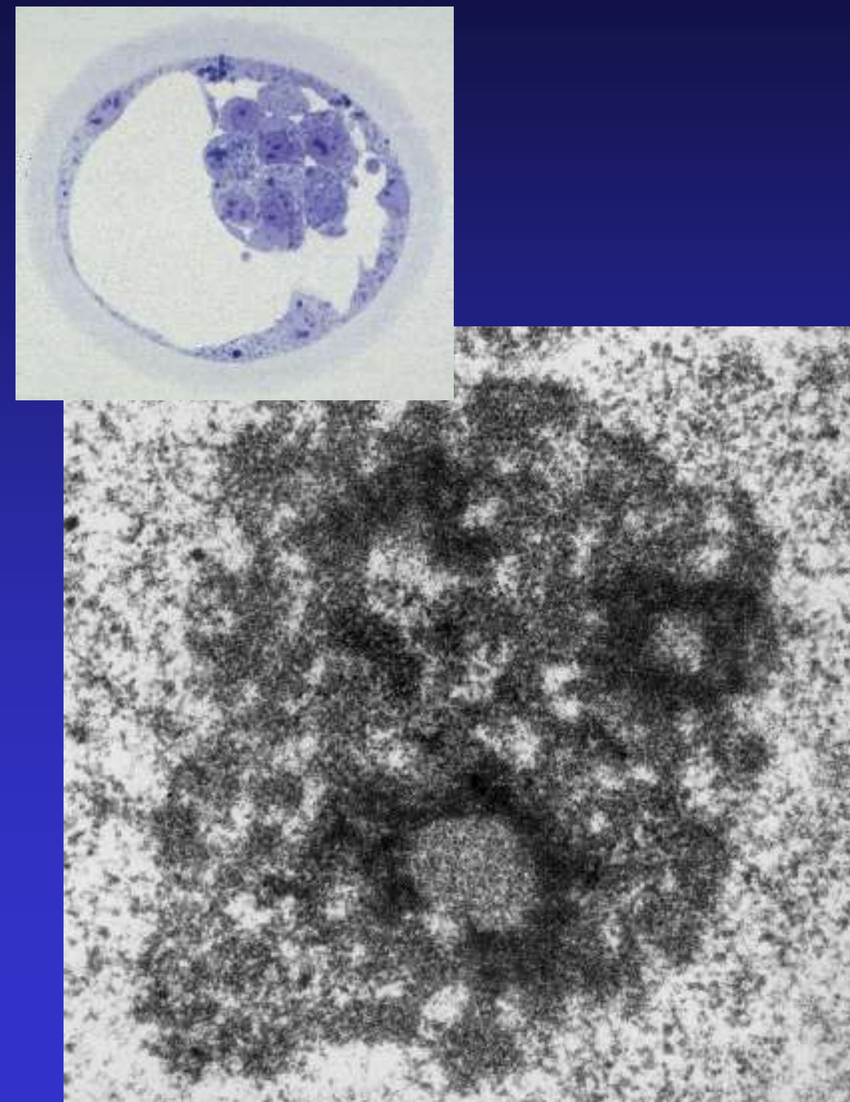
Ultrastructure of the bovine blastocyst nucleolus



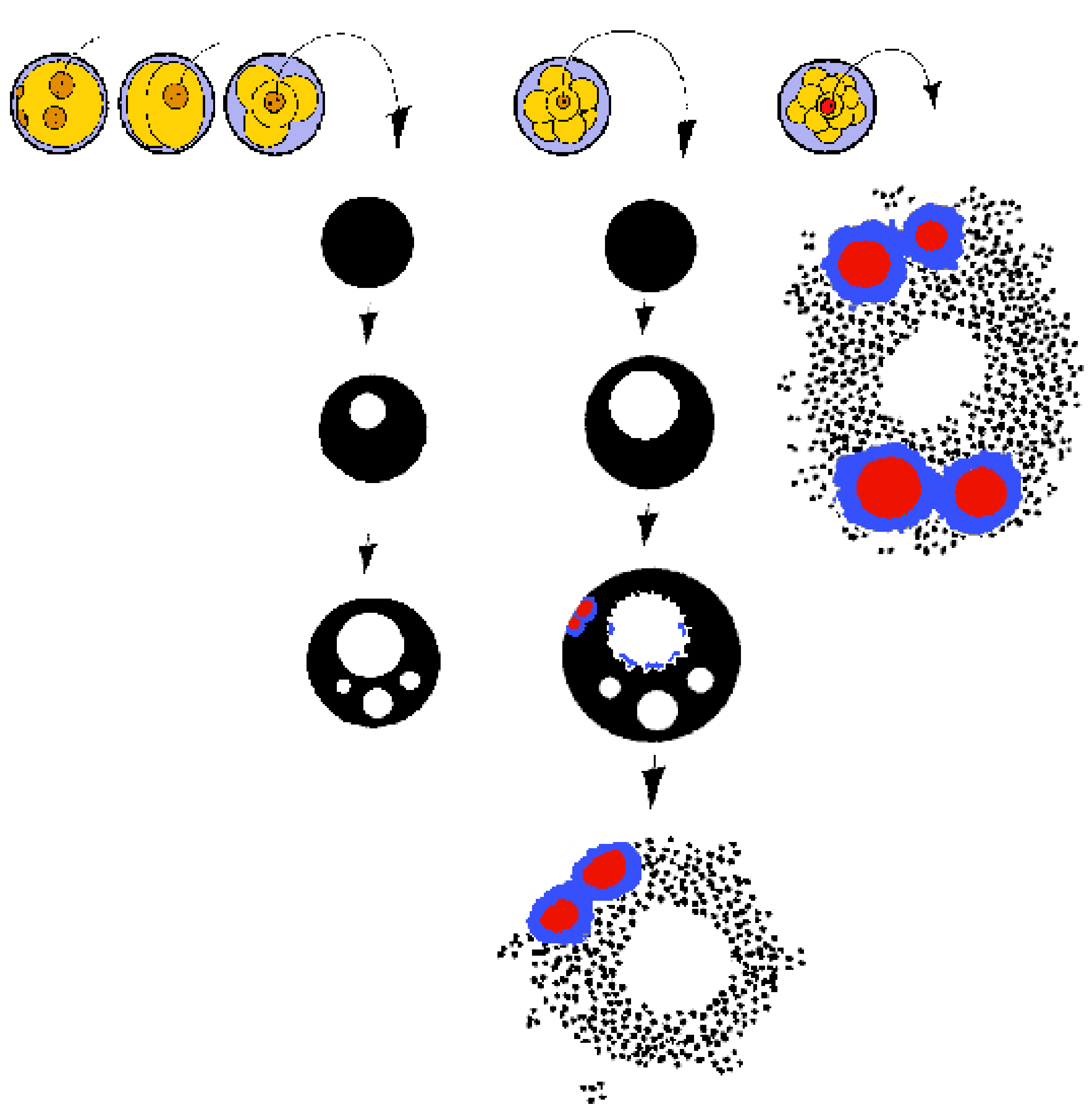


rRNA genes as a marker for embryonic gene activation



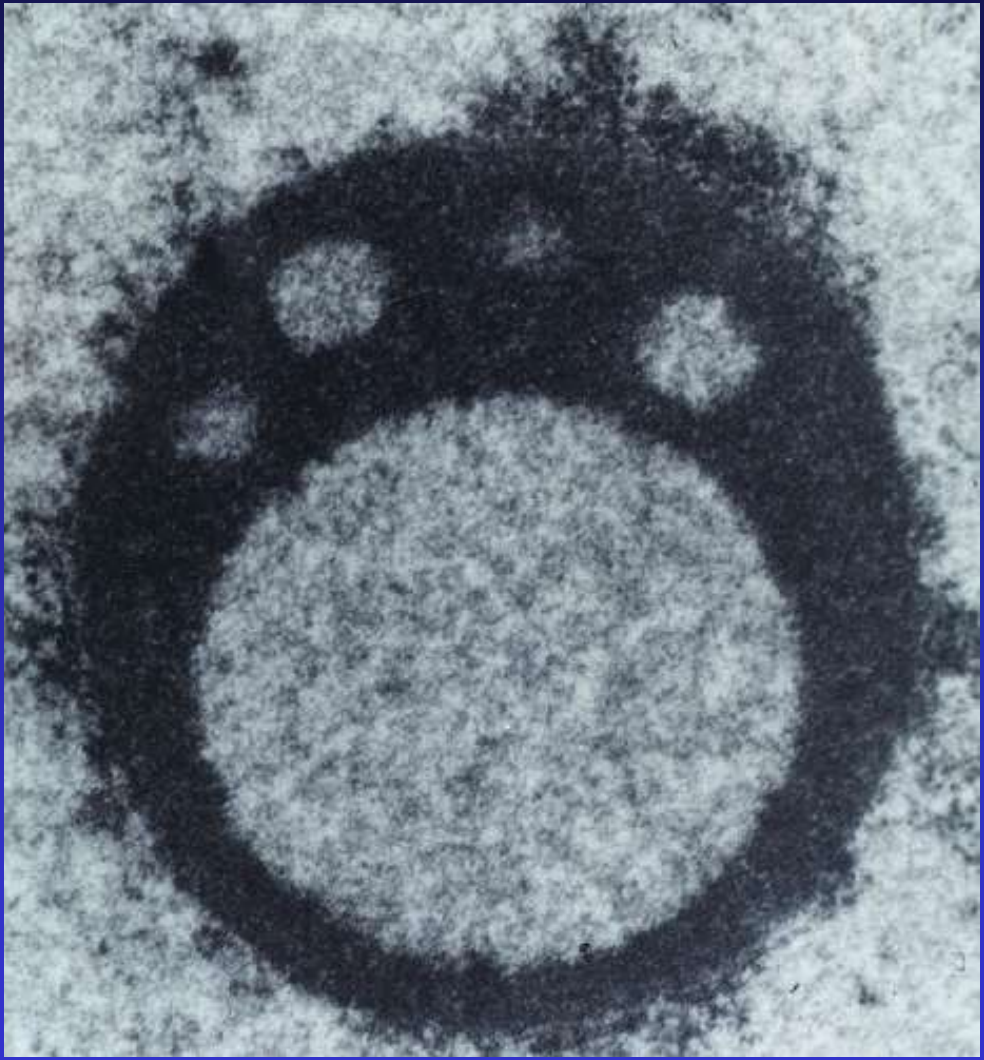


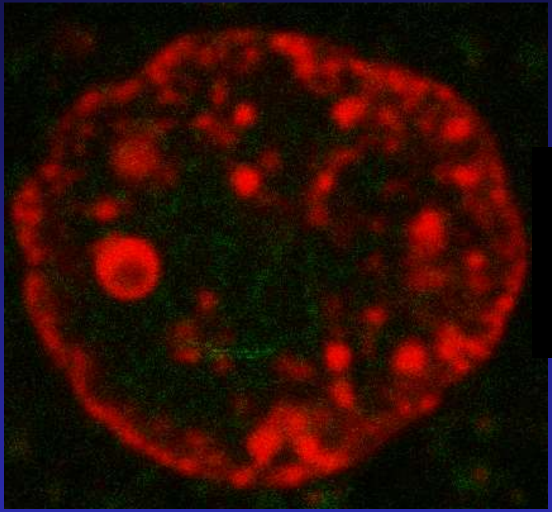
Nucleolar ultrastructure in bovine embryos



Nucleolar proteins in bovine embryos

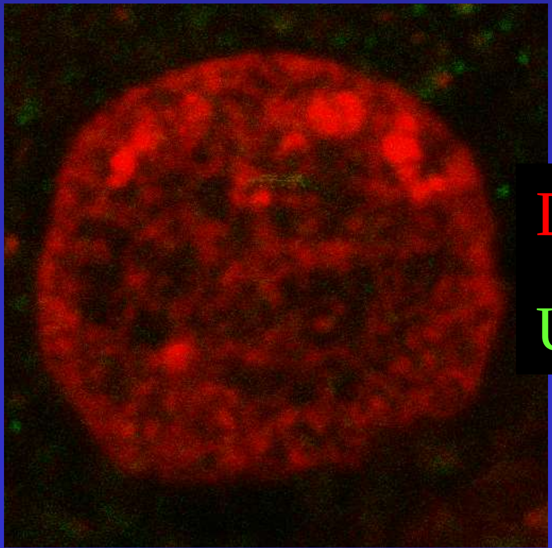
4th cell cycle, 8-cell stage - Early





DNA

RNA pol I

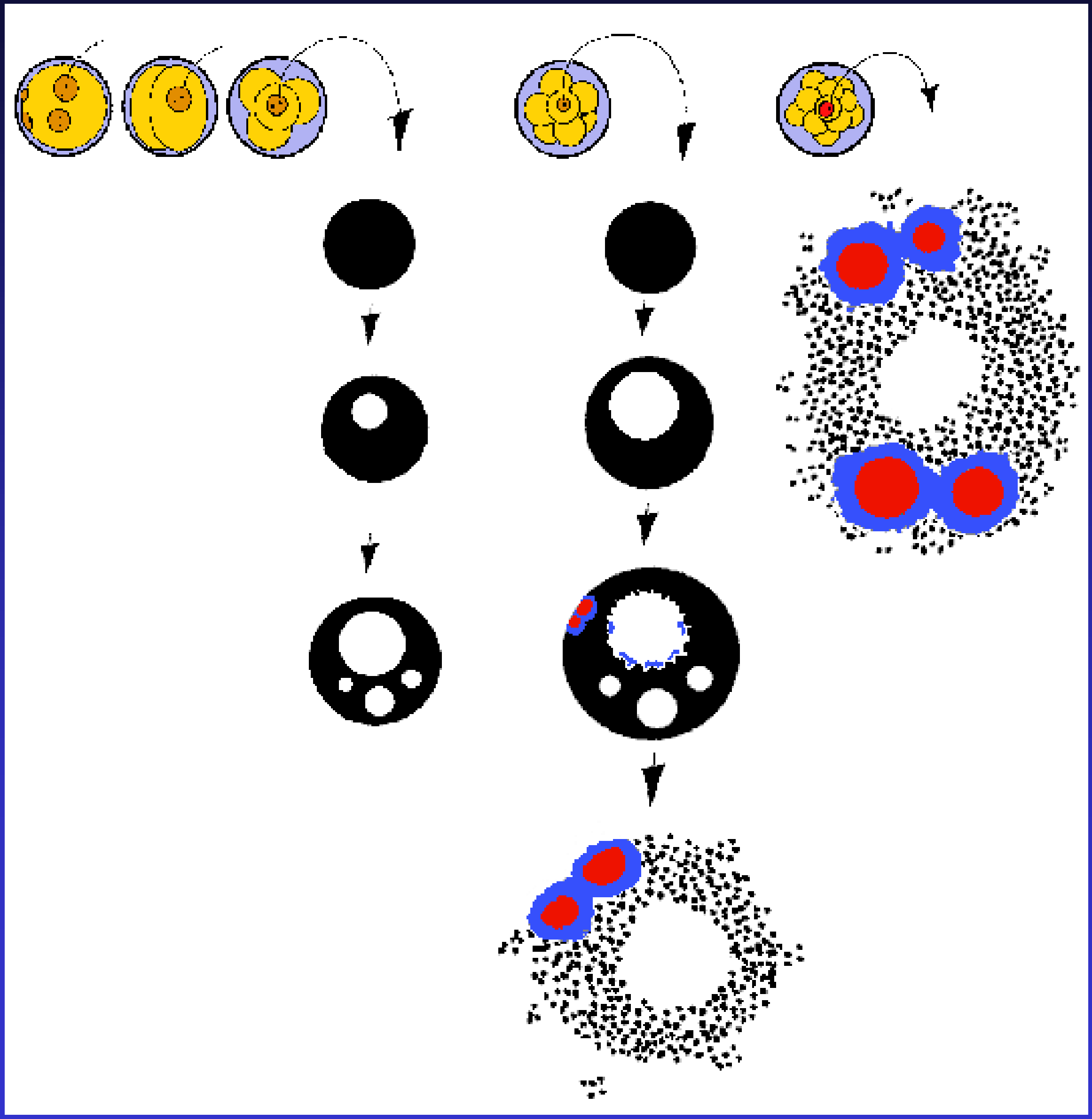


DNA

UBF

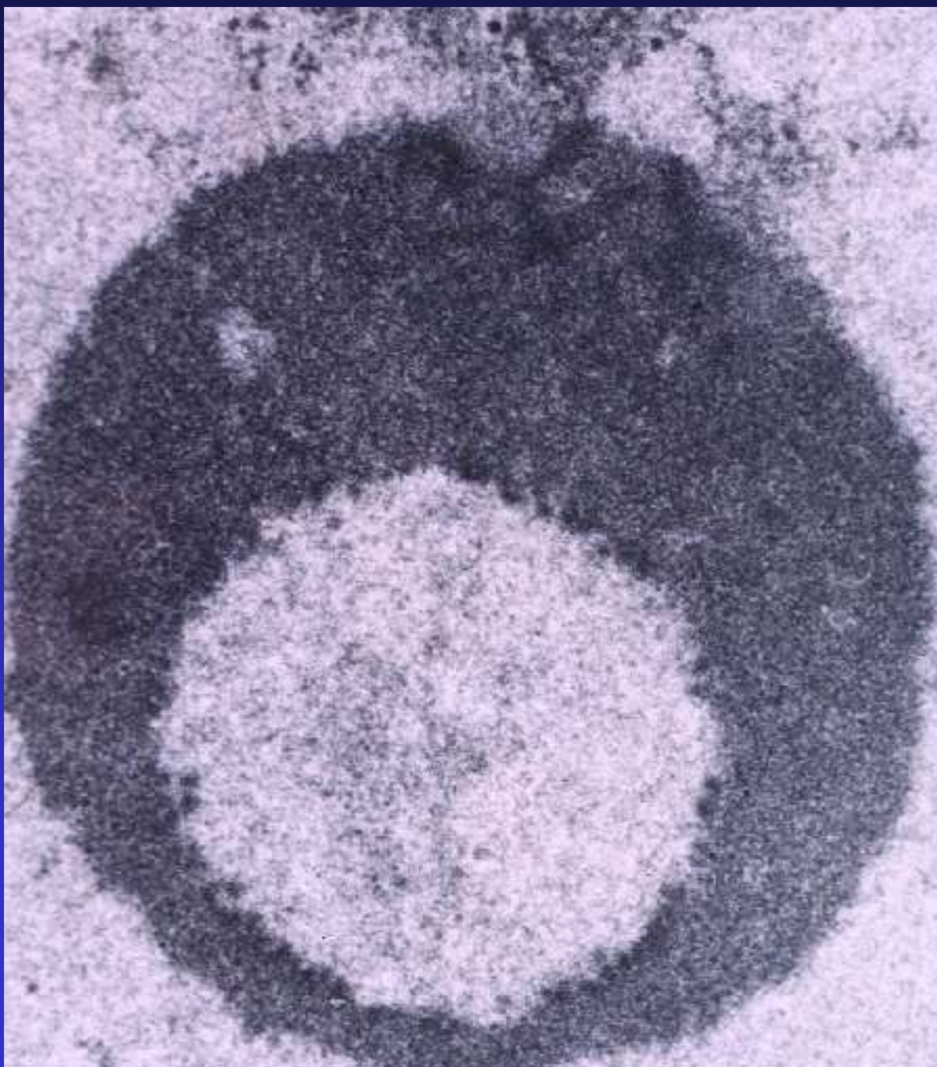
Upstream binding factor

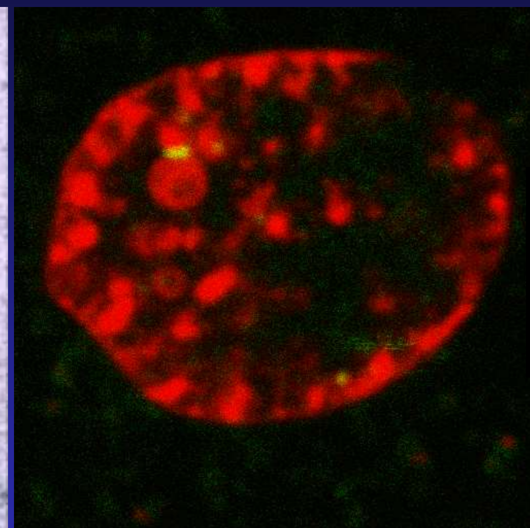
Nucleolar ultrastructure in bovine embryos



Nucleolar proteins in bovine embryos

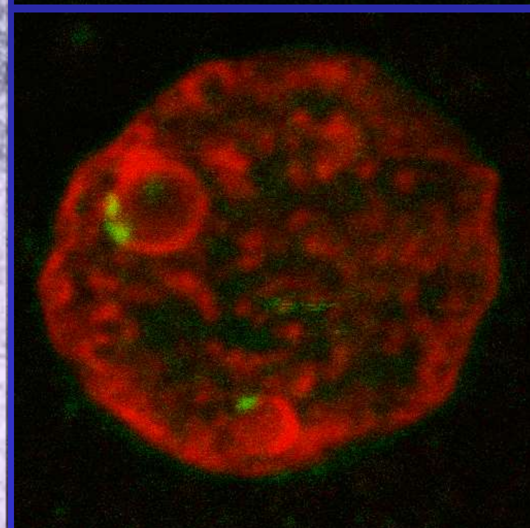
4th cell cycle, 8-cell stage - Late





DNA

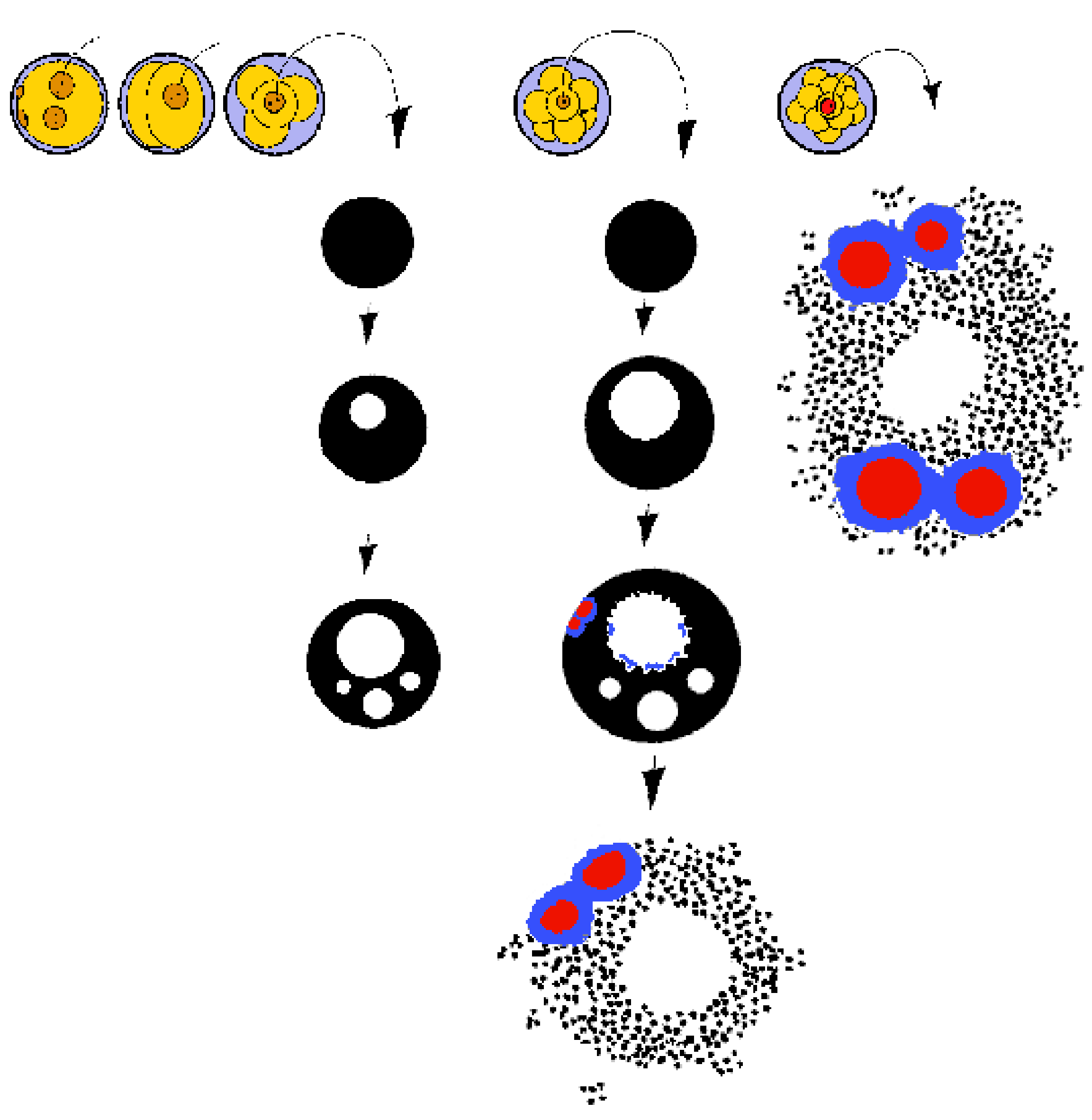
RNA pol I



DNA

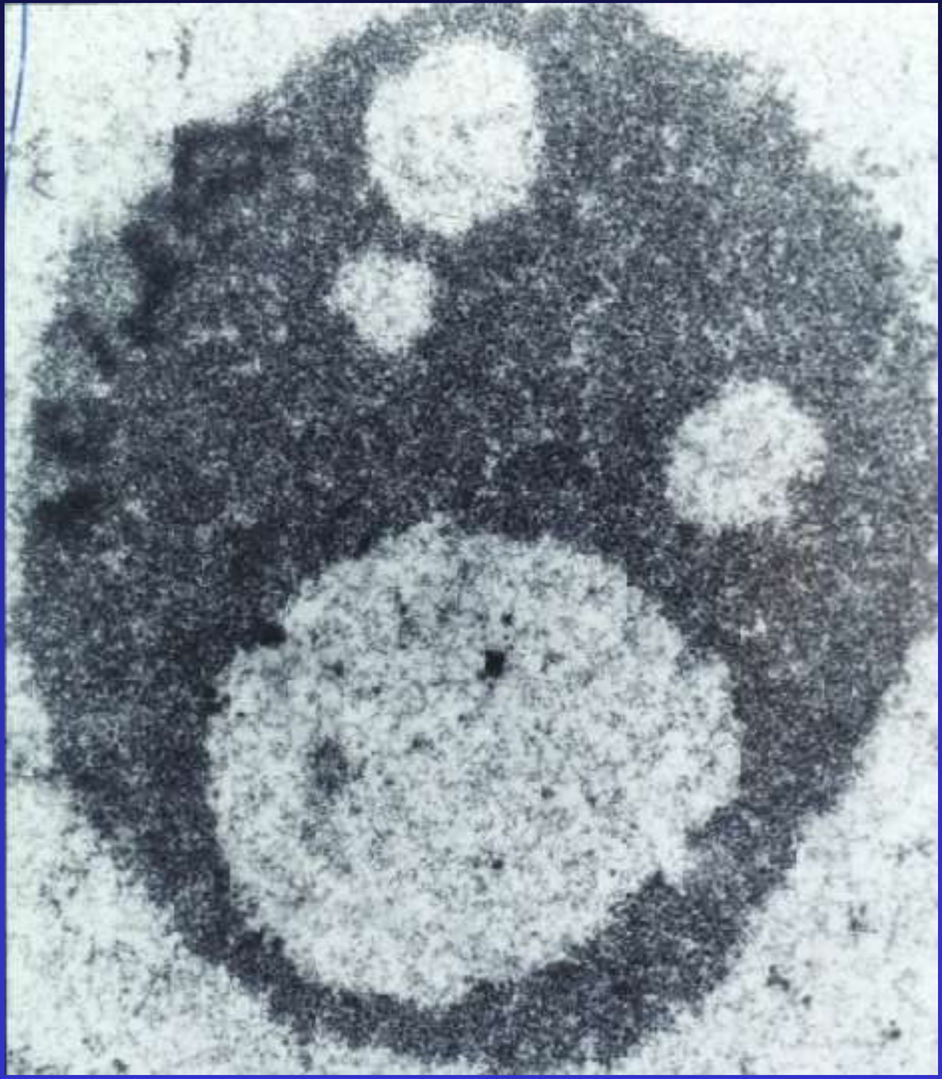
UBF

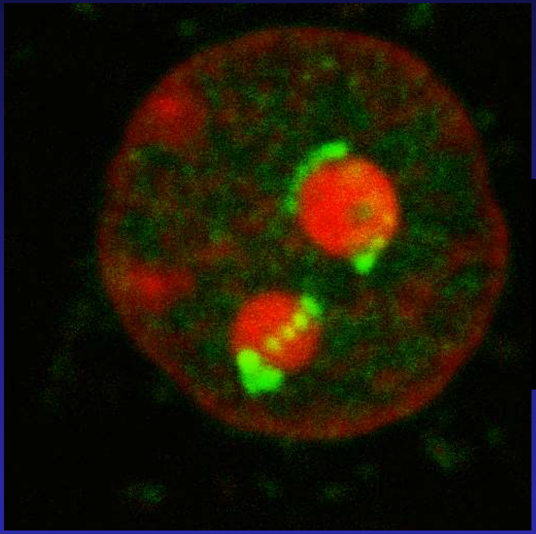
Nucleolar ultrastructure in bovine embryos



Nucleolar proteins in bovine embryos

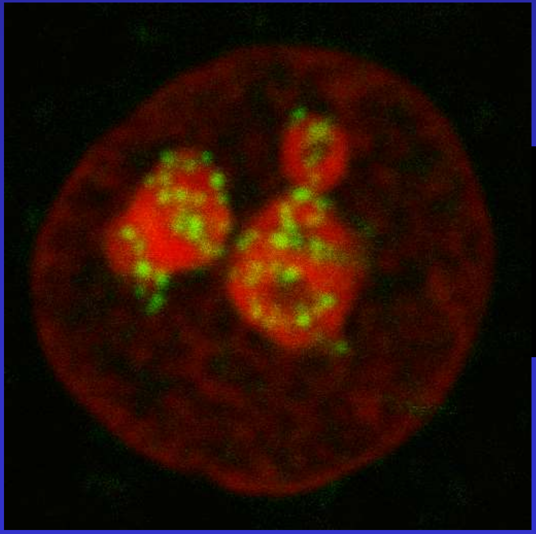
5th cell cycle, 16-cell stage





DNA

RNA pol I



DNA

UBF

Nucleolar proteins in bovine embryos

Single nuclei from bovine embryos

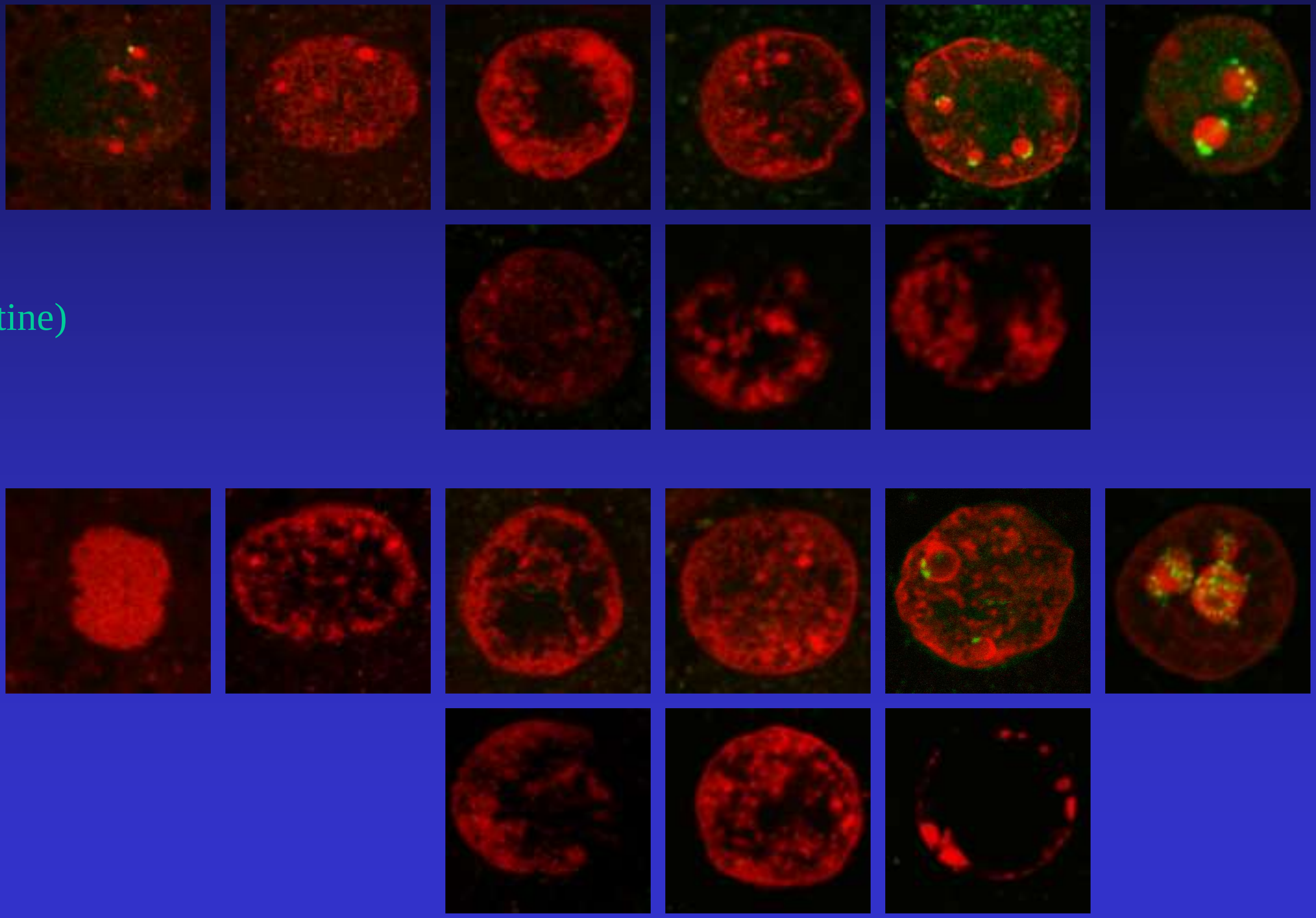
1-cell 2-cell 4-cell 8-cell-e 8-cell-l 16-cell

RNA pol I

RNA pol I (amanitine)

UBF

UBF (amanitine)



Jiří K a ň k a

Embryonální kmenové buňky savců

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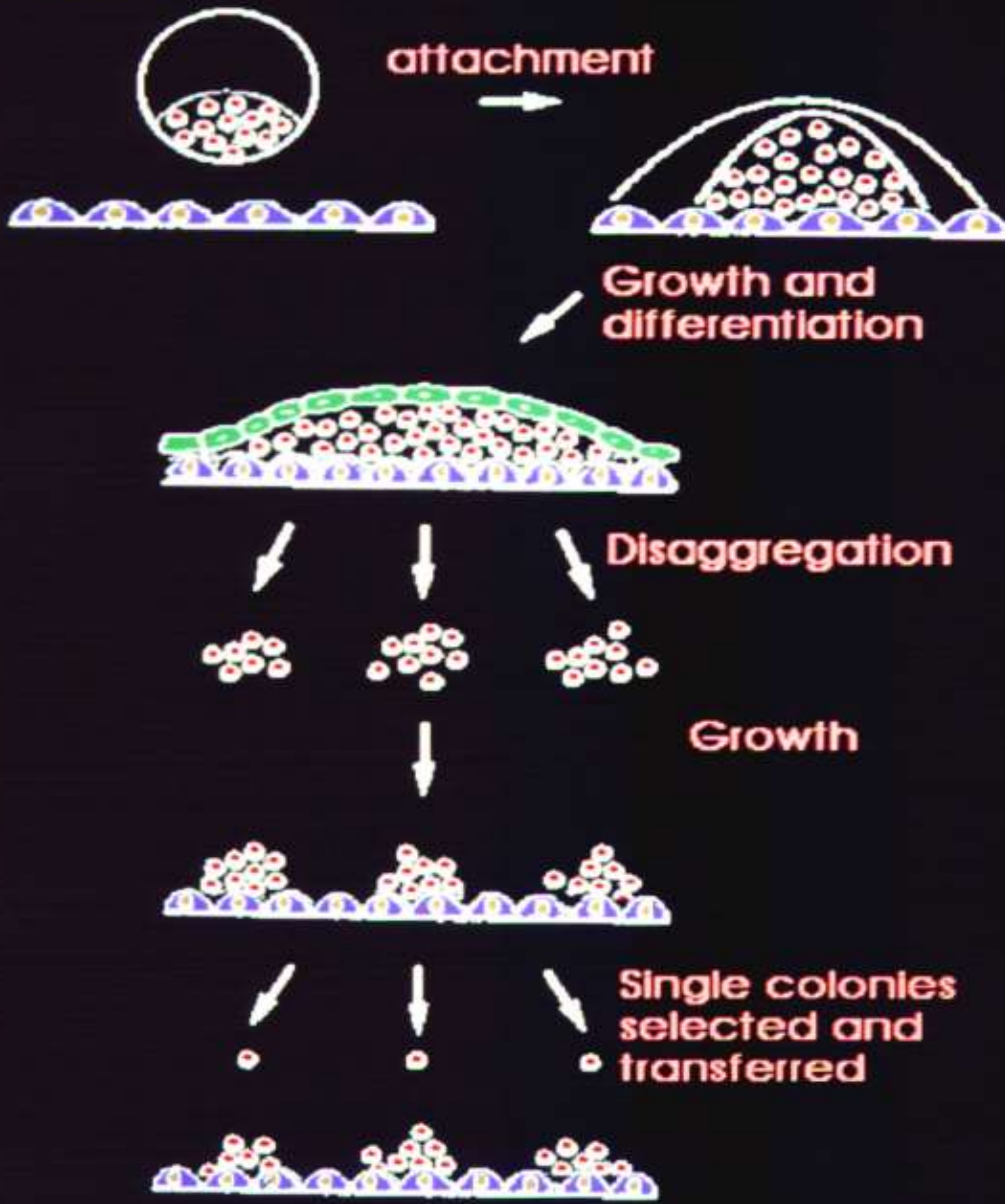
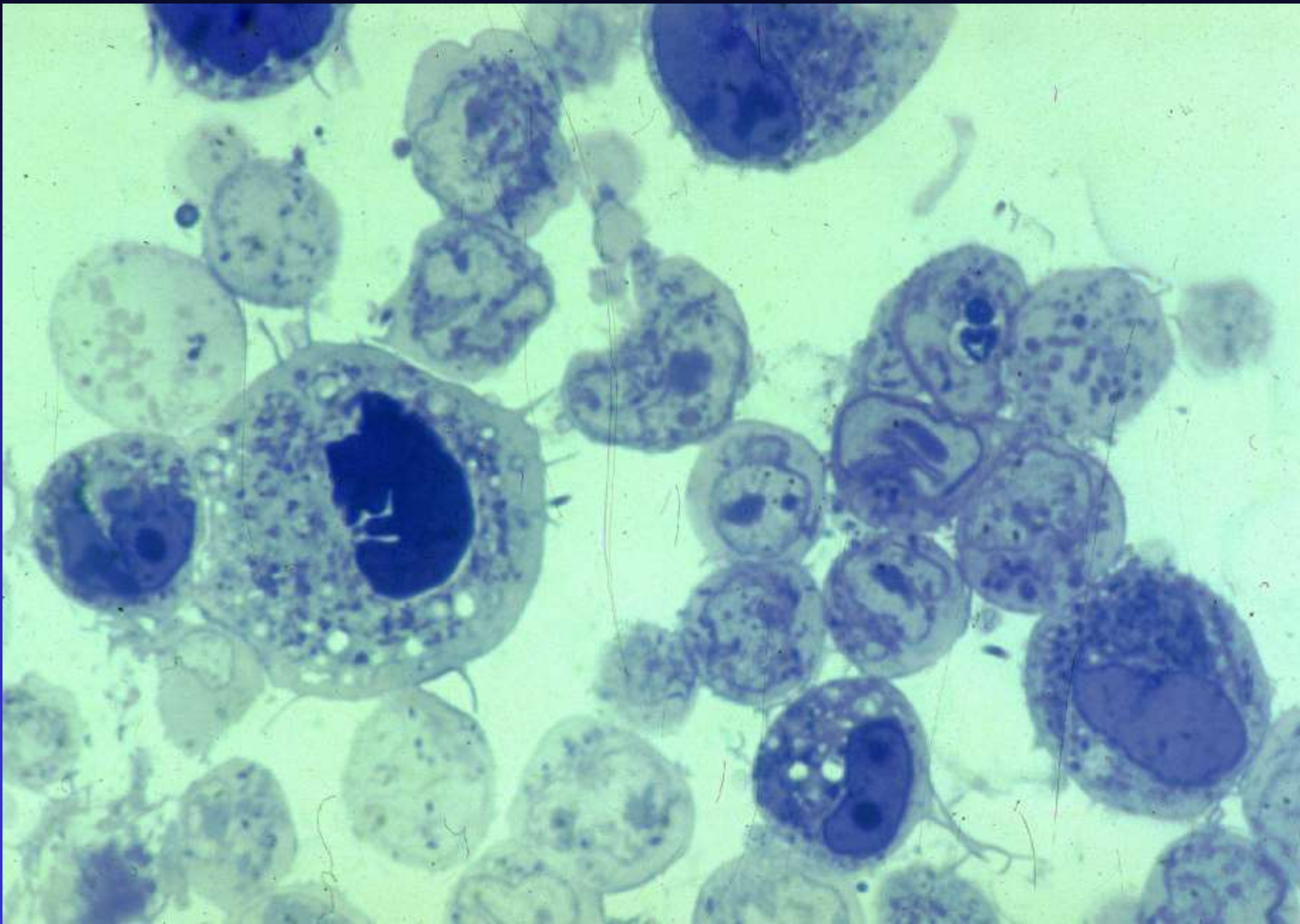
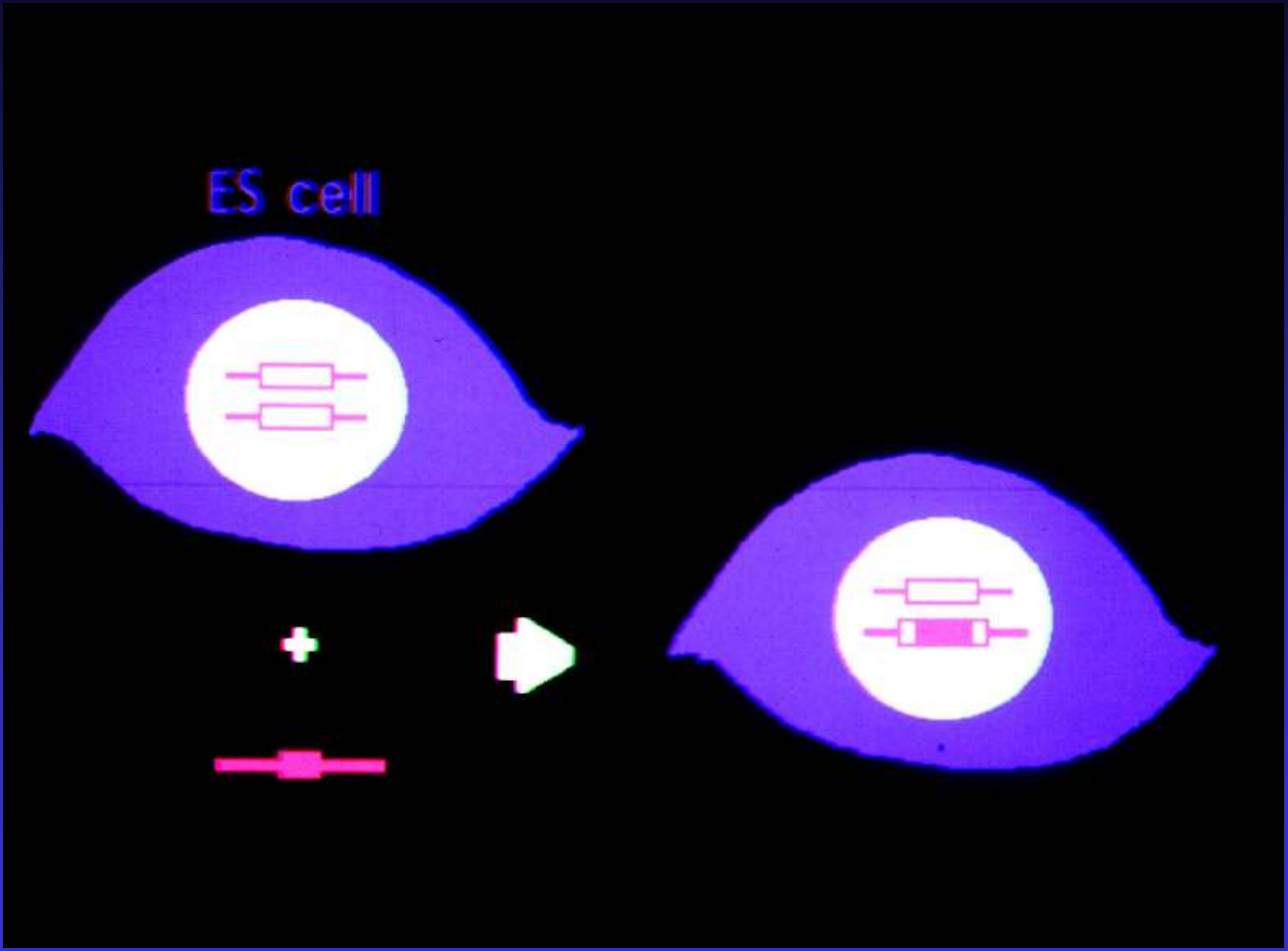
Isolation of embryo-derived stem cells






ES cell

EMBRYONIC STEM CELLS

STEVENS (1959, 1964) TERATOCARCINOMA
PIERCE (1960) EC
KLEINSMITH, PIERCE (1964) EC
STEWART, MINTZ (1981)

EVANS, KAUFMAN (1981) ES
MARTIN (1981)
SMITH, HOOPER (1987) - FEEDER CELLS, LIF
FOLGER, THOMAS, CAPECCHI (1982) - HOMOLOGOUS RECOMBINATION
THOMSON (1989) - GENE TARGETING, GERM LINE
SIMS, FIRST (1993) - CLONING, FARM ANIMAL
STEWART et al. (1994), MATSUI (1992) – GERM CELLS
J. GEARHART´S GROUP (SHAMBLOTT et al., 1998) – HUMAN EG
UNIV. OF WISCONSIN (THOMSON et al., 1998) – HUMAN ES

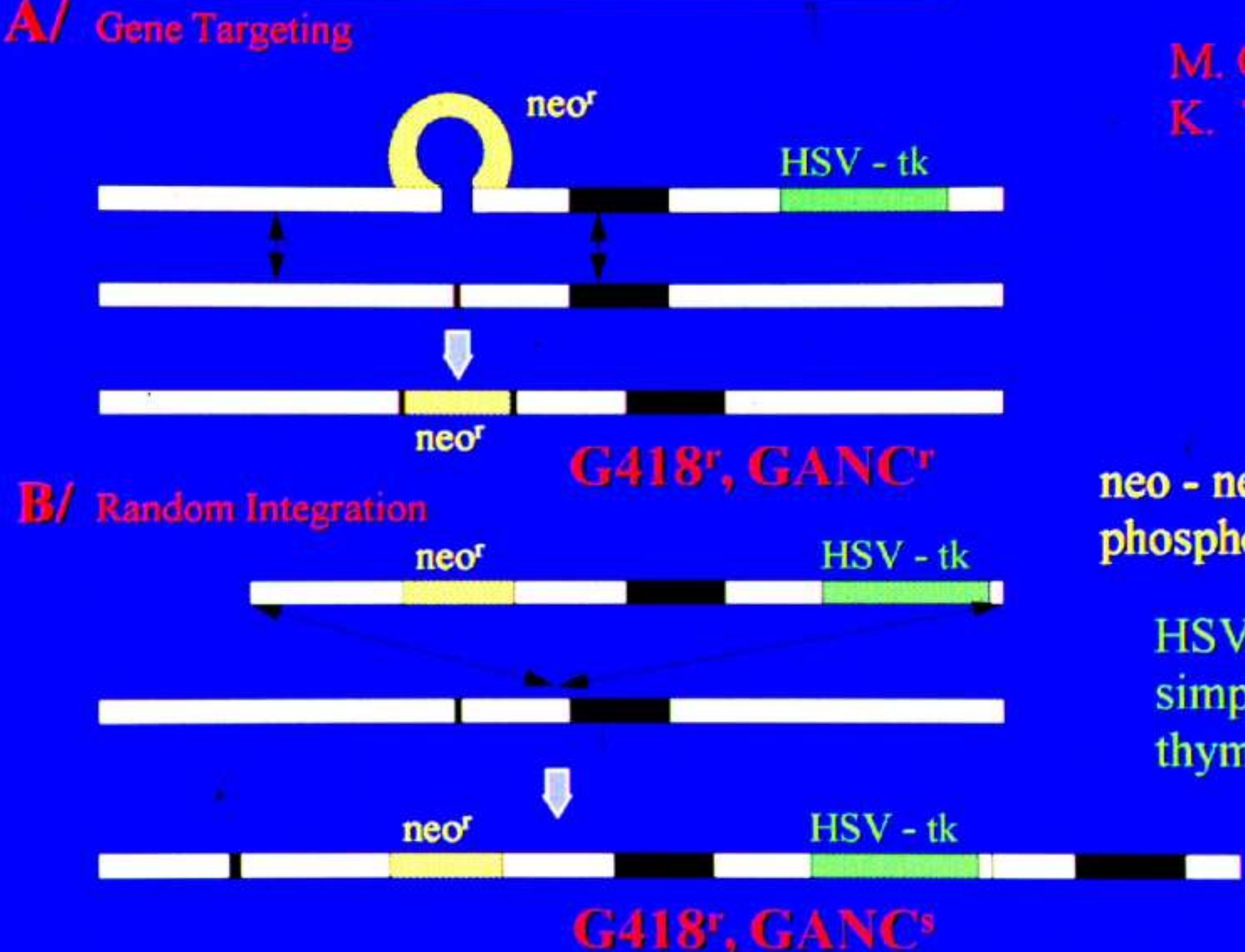
POUŽITÍ EMBRYONÁLNÍCH KMENOVÝCH BUNĚK

GENE TARGETING EXP.
SUBTLE MUTATION (HIT AND RUN)
CRE RECOMBINASE

GENE TRAP
ENHARCER TRAP
CLONING

POSITIVE - NEGATIVE SELECTION

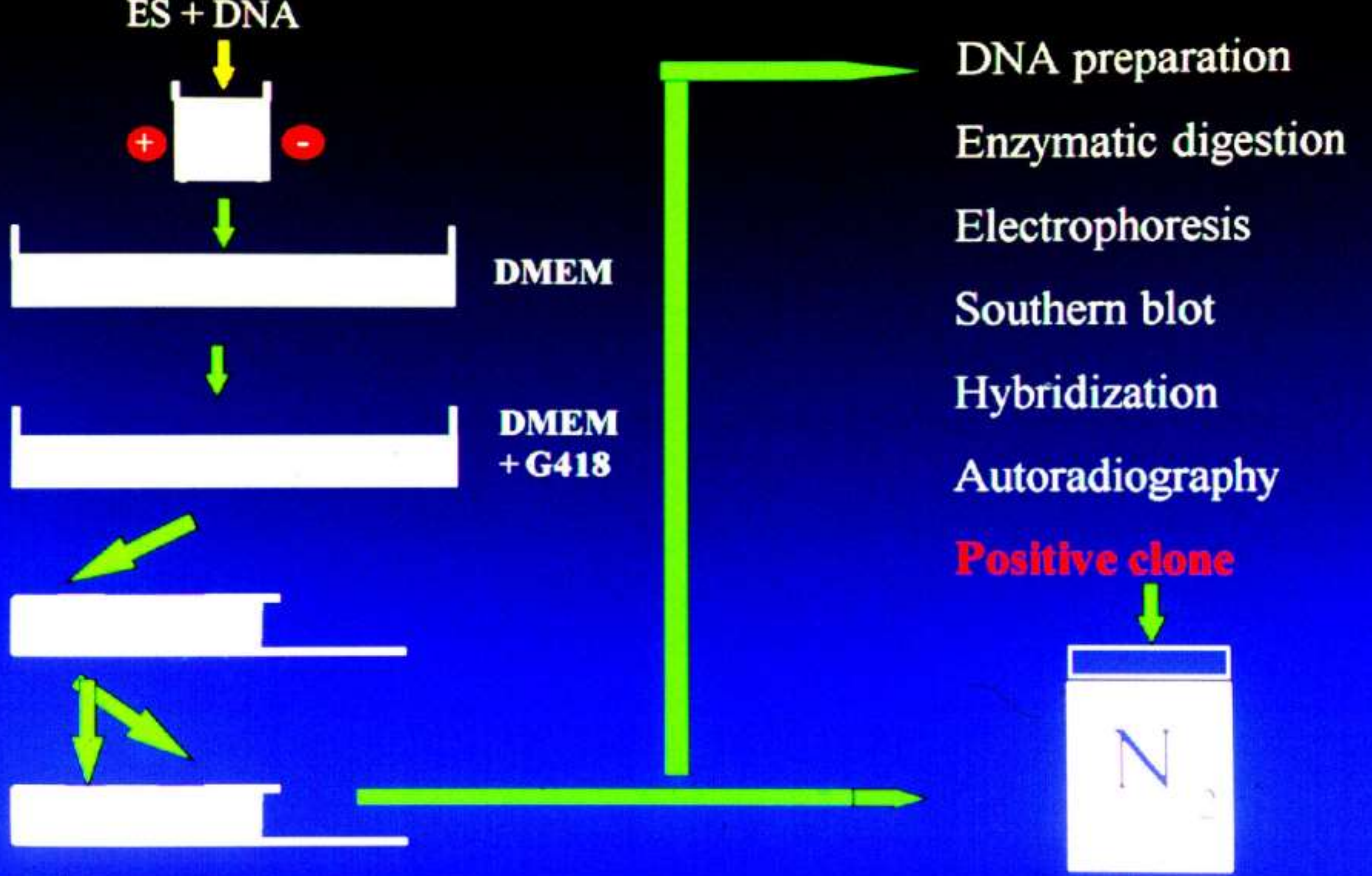
M. Capecchi,
K. Thomas



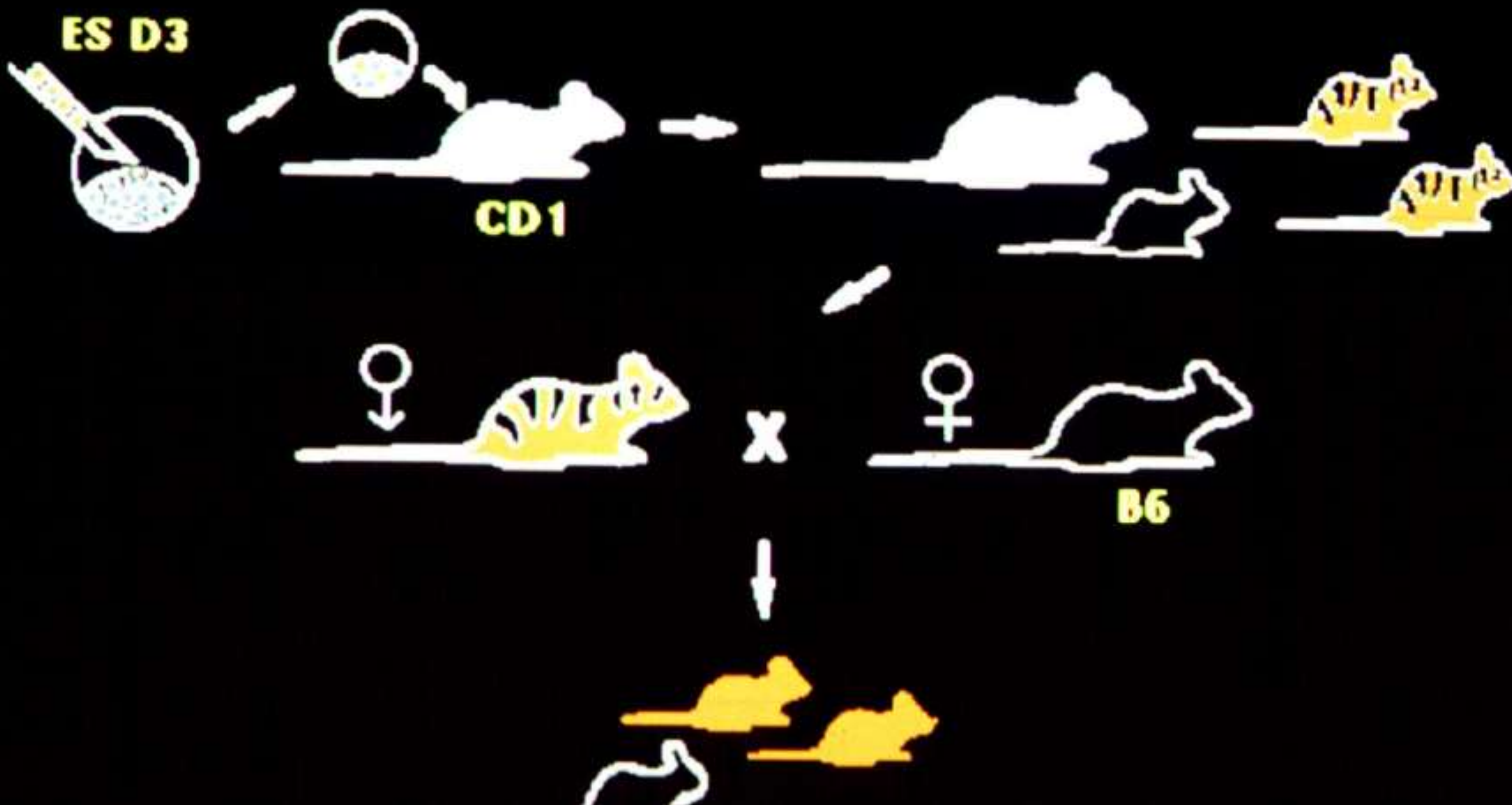
neo - neomycin-phosphotransferase

HSV-tk - Herpes simplex virus thymidine kinase

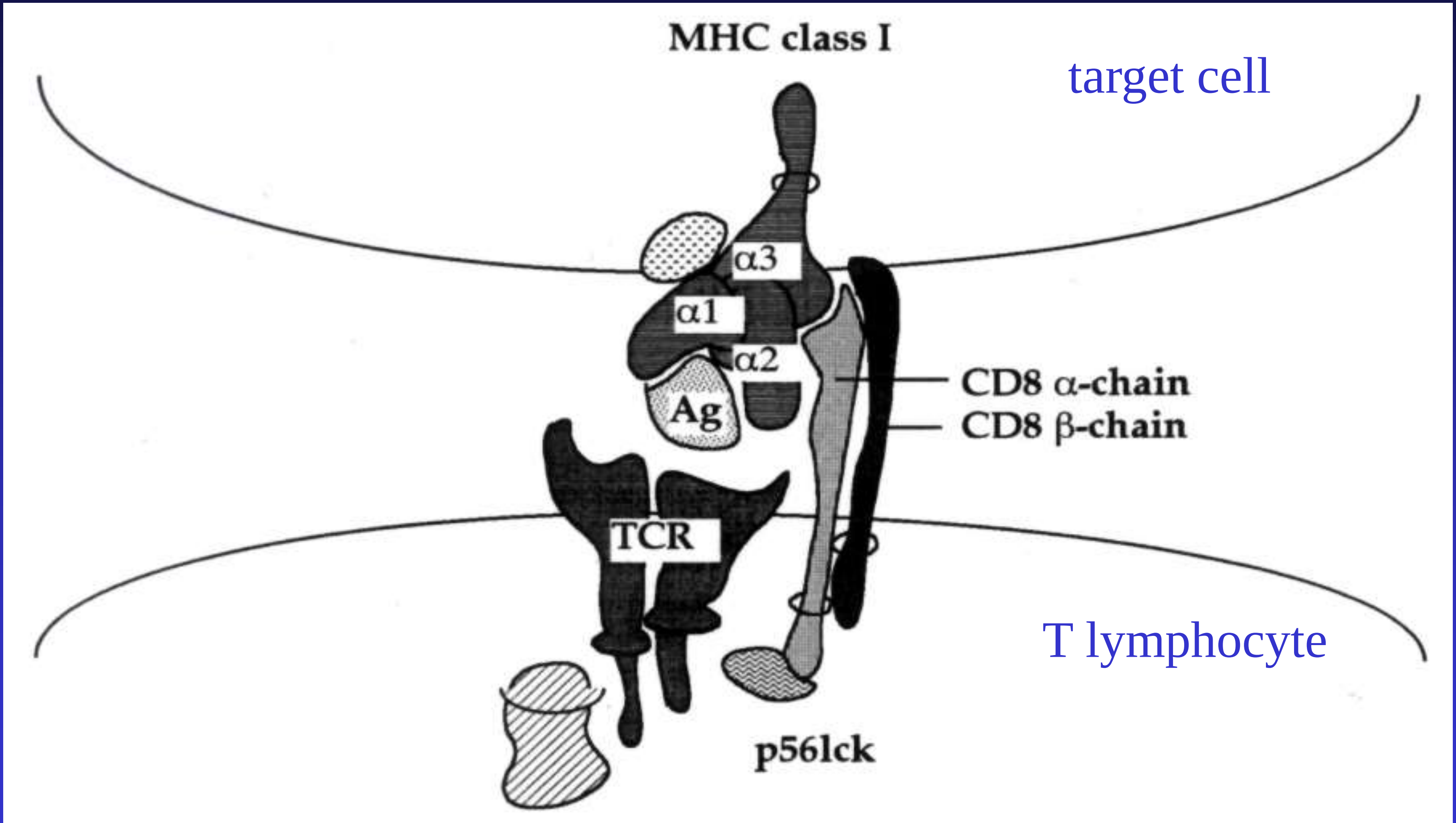
General selection strategy for homologous recombination

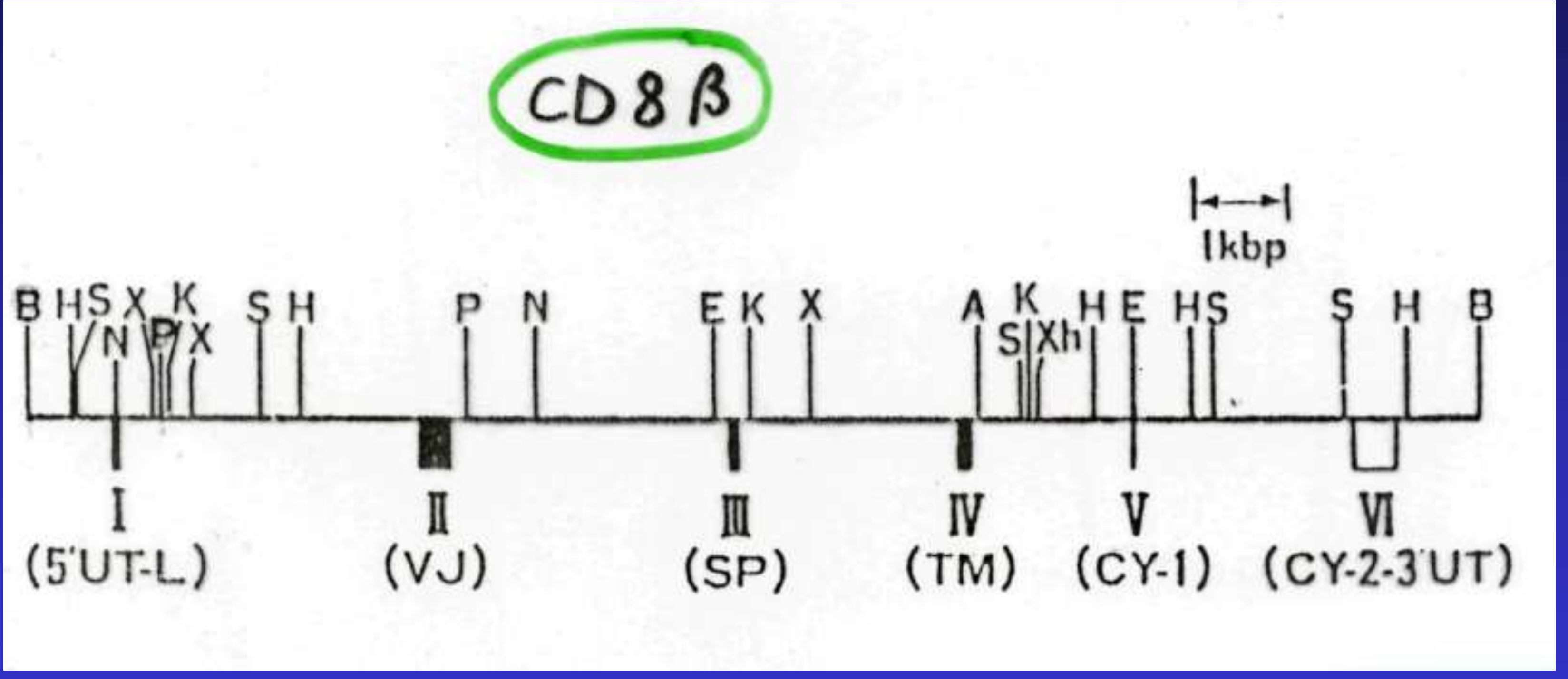


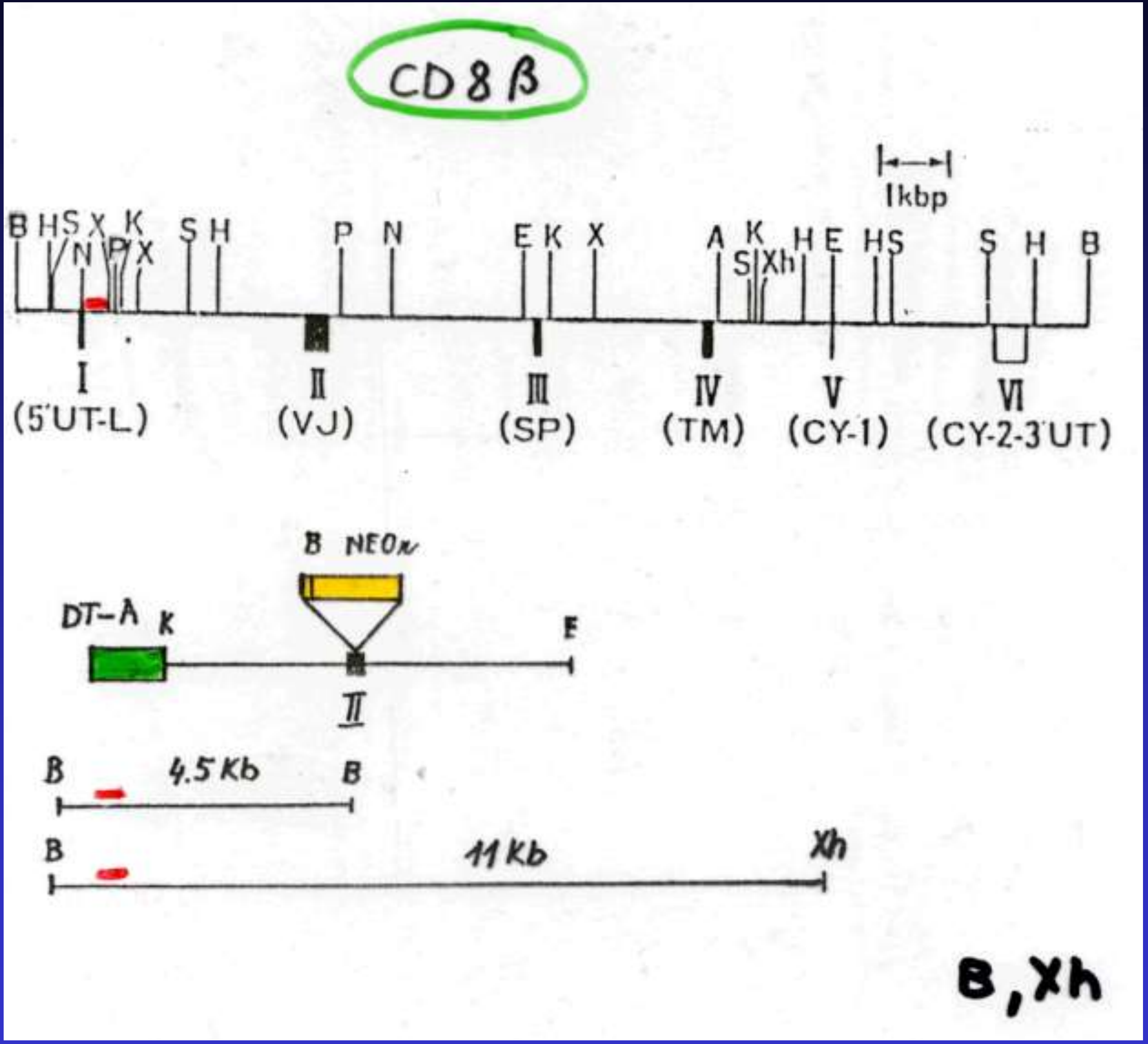
Generation of mouse germ-line chimeras

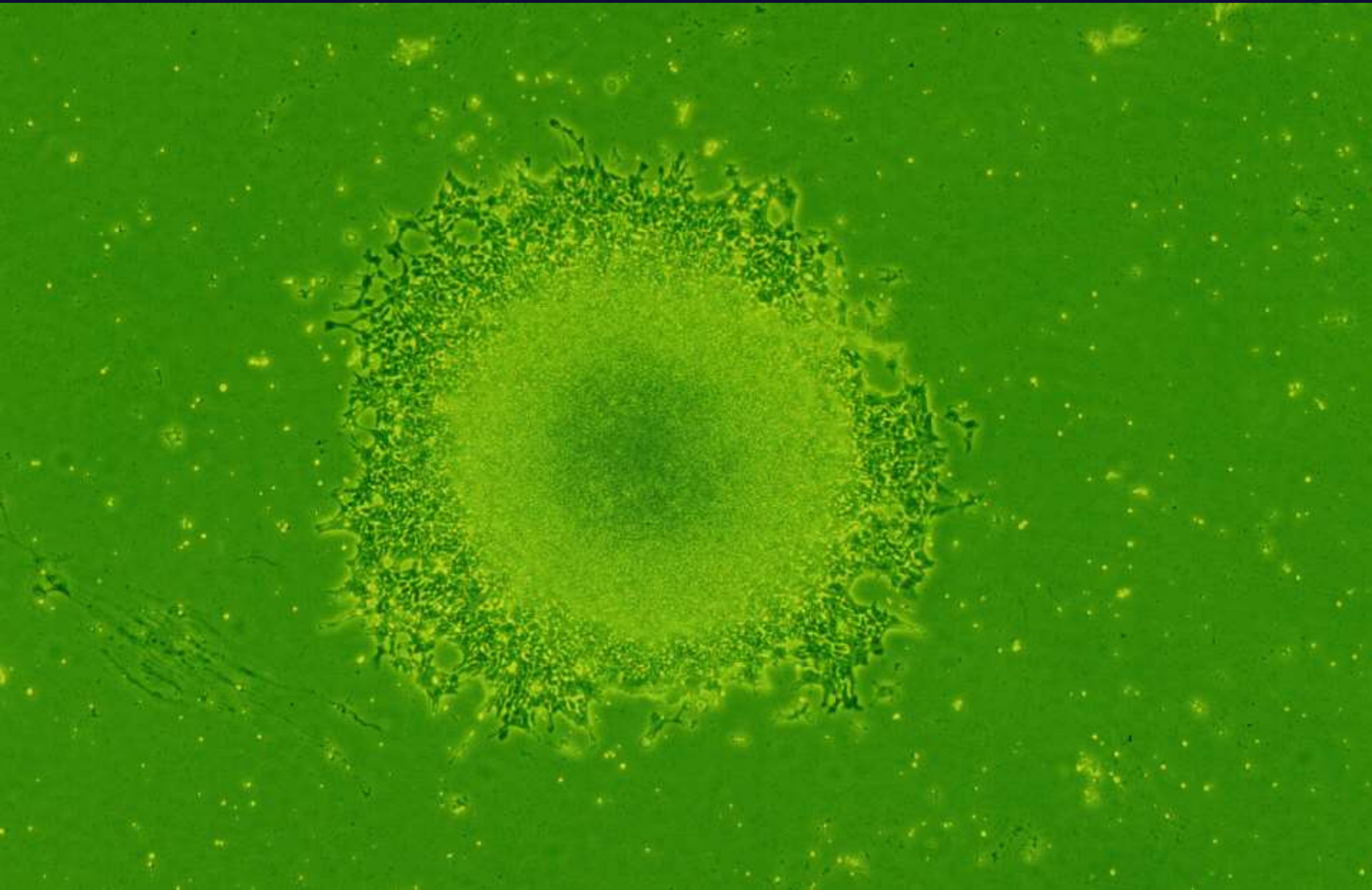


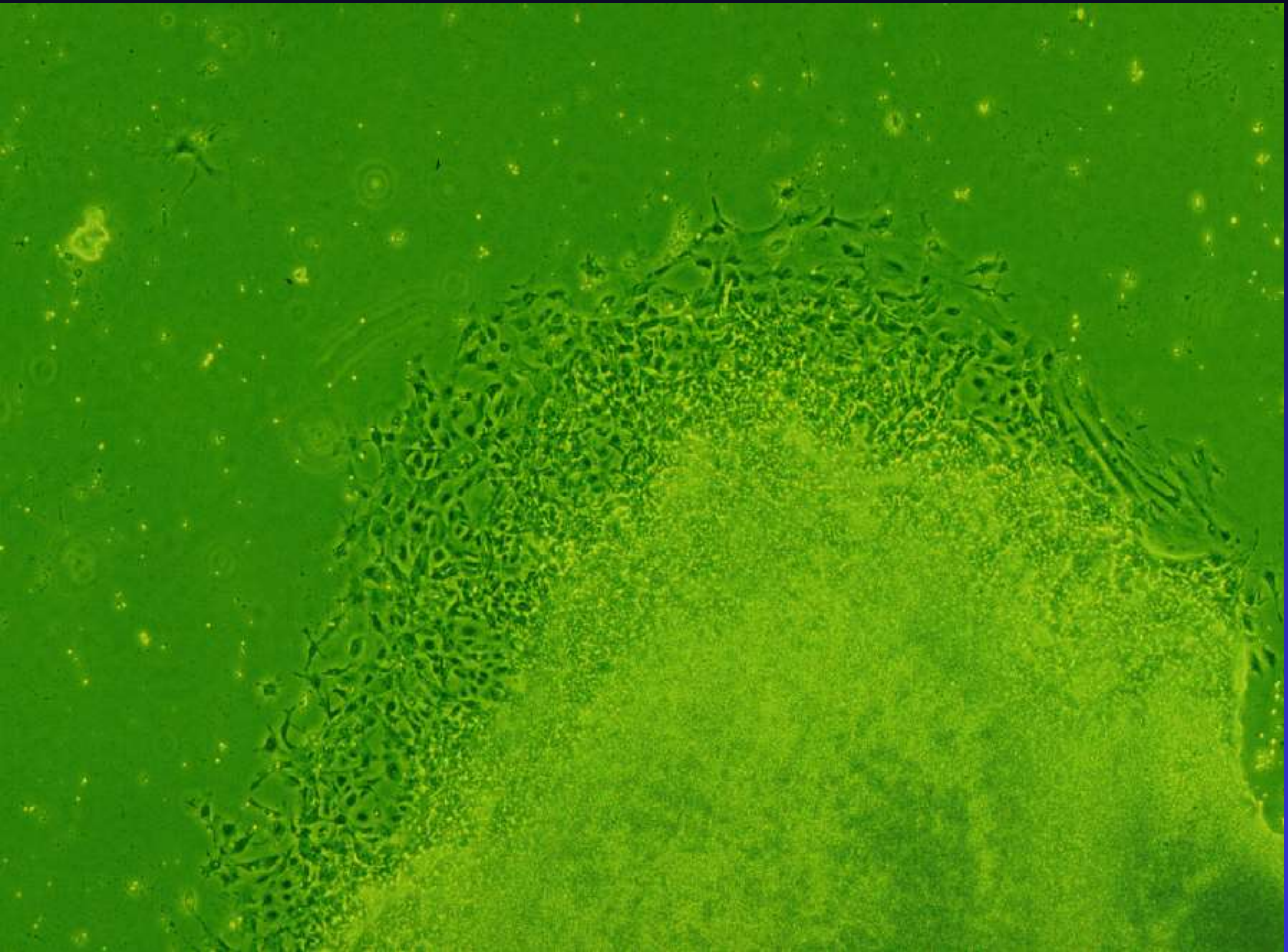
Gene targeting of CD8 beta

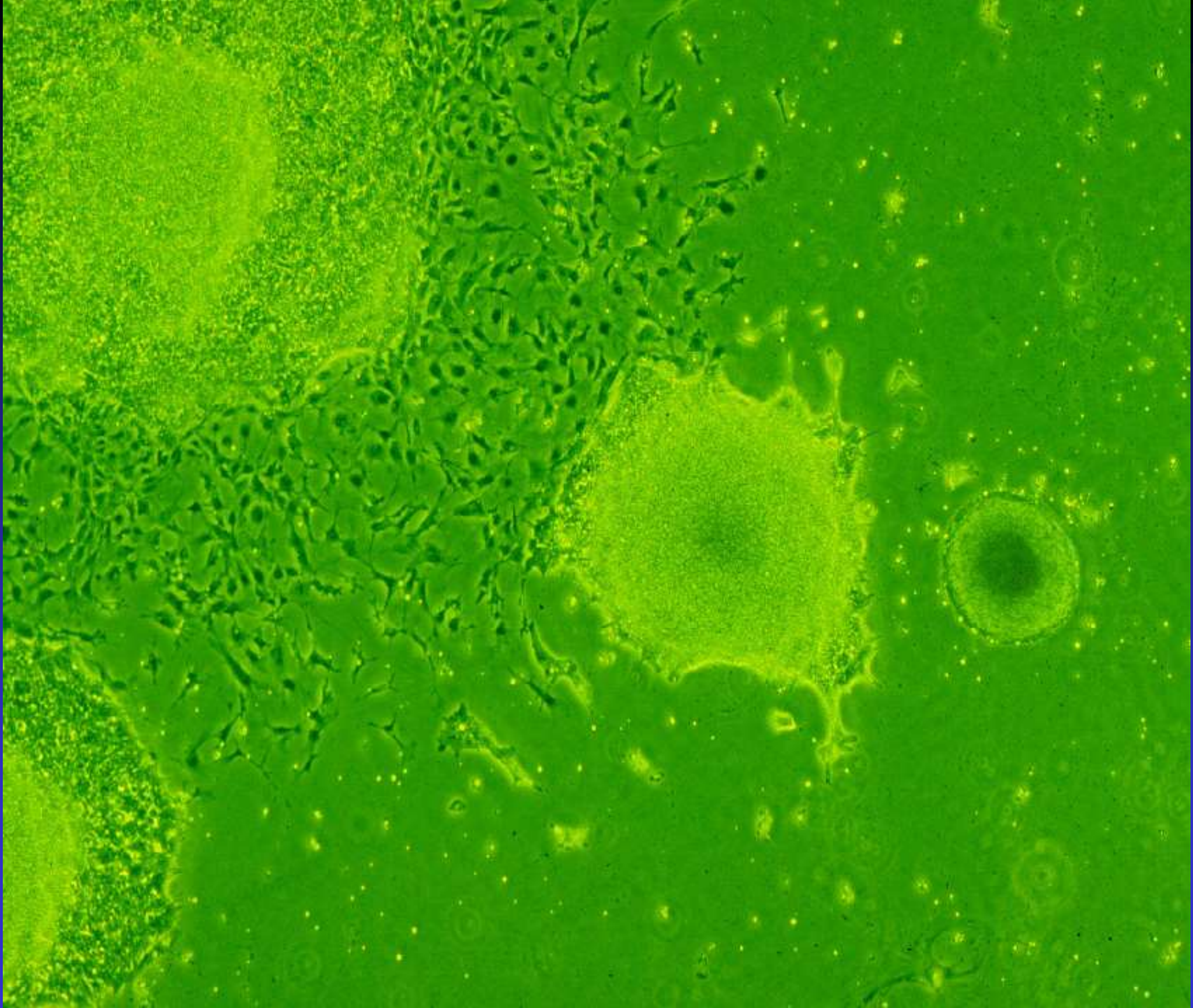



CD8β
1kbp
B H S X K S H P N E K X A K H E H S S H B
N P X S Xh
I
(5'UT-L)
II
(VJ)
III
(SP)
IV
(TM)
V
(CY-1)
VI
(CY-2-3'UT)

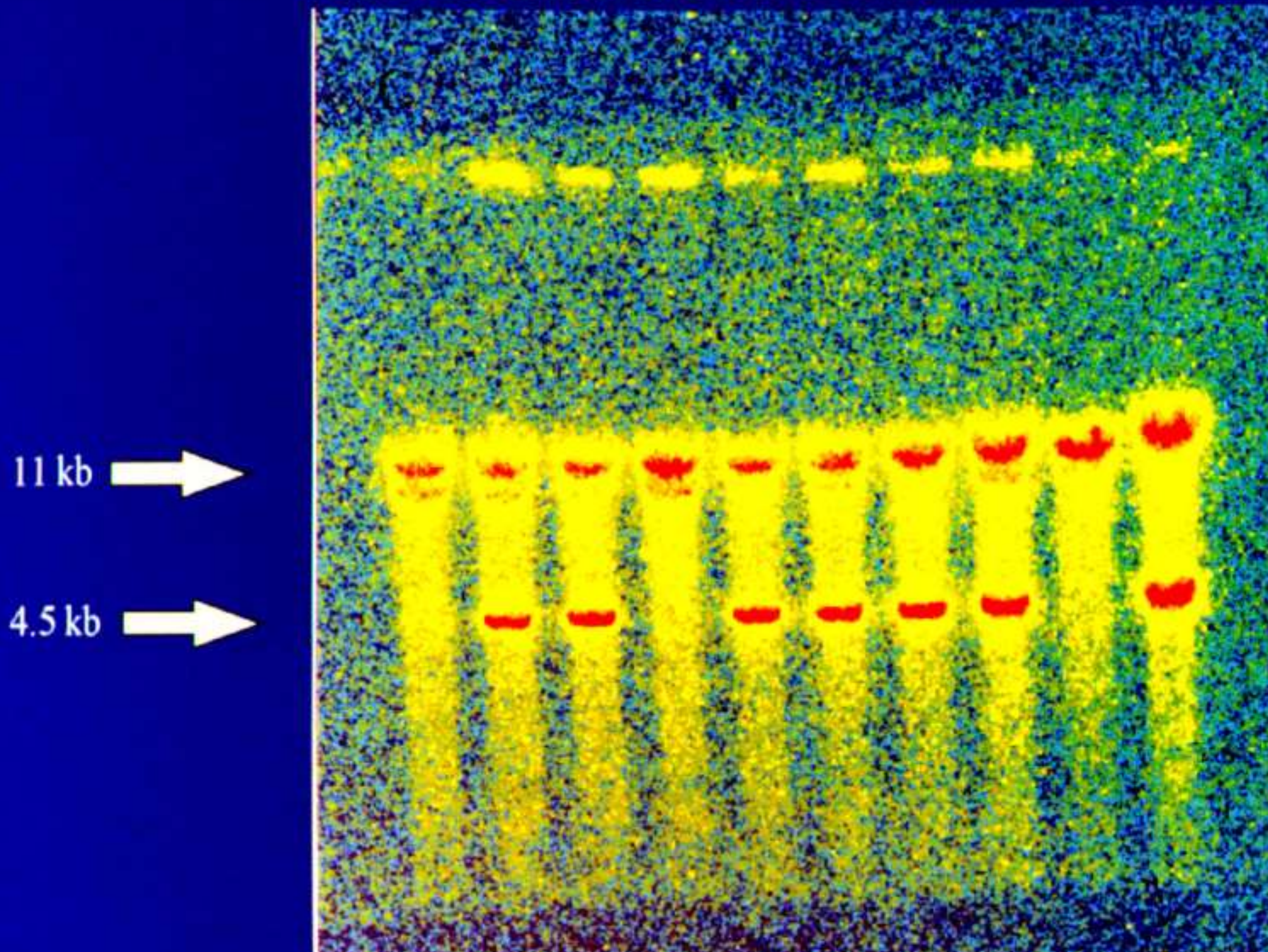

CD8 β
1kbp
B H S X K N P X S H P N E K X A K S Xh H E H S S H B
I (5'UT-L)
II (VJ)
III (SP)
IV (TM)
V (CY-1)
VI (CY-2-3'UT)
B NEOR
DT-A K
E
II
B 4.5 Kb B
B 11 Kb Xh
B, Xh

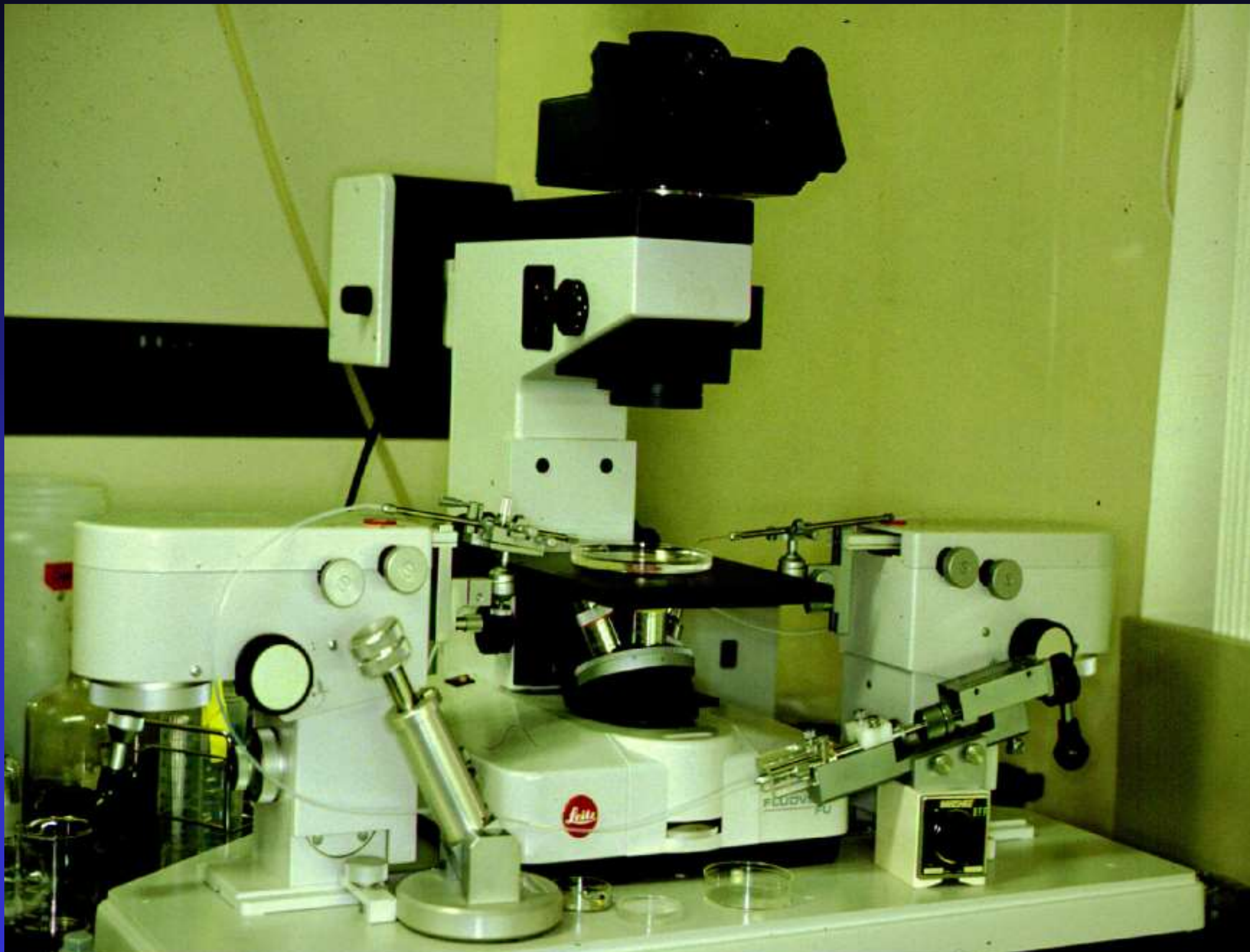




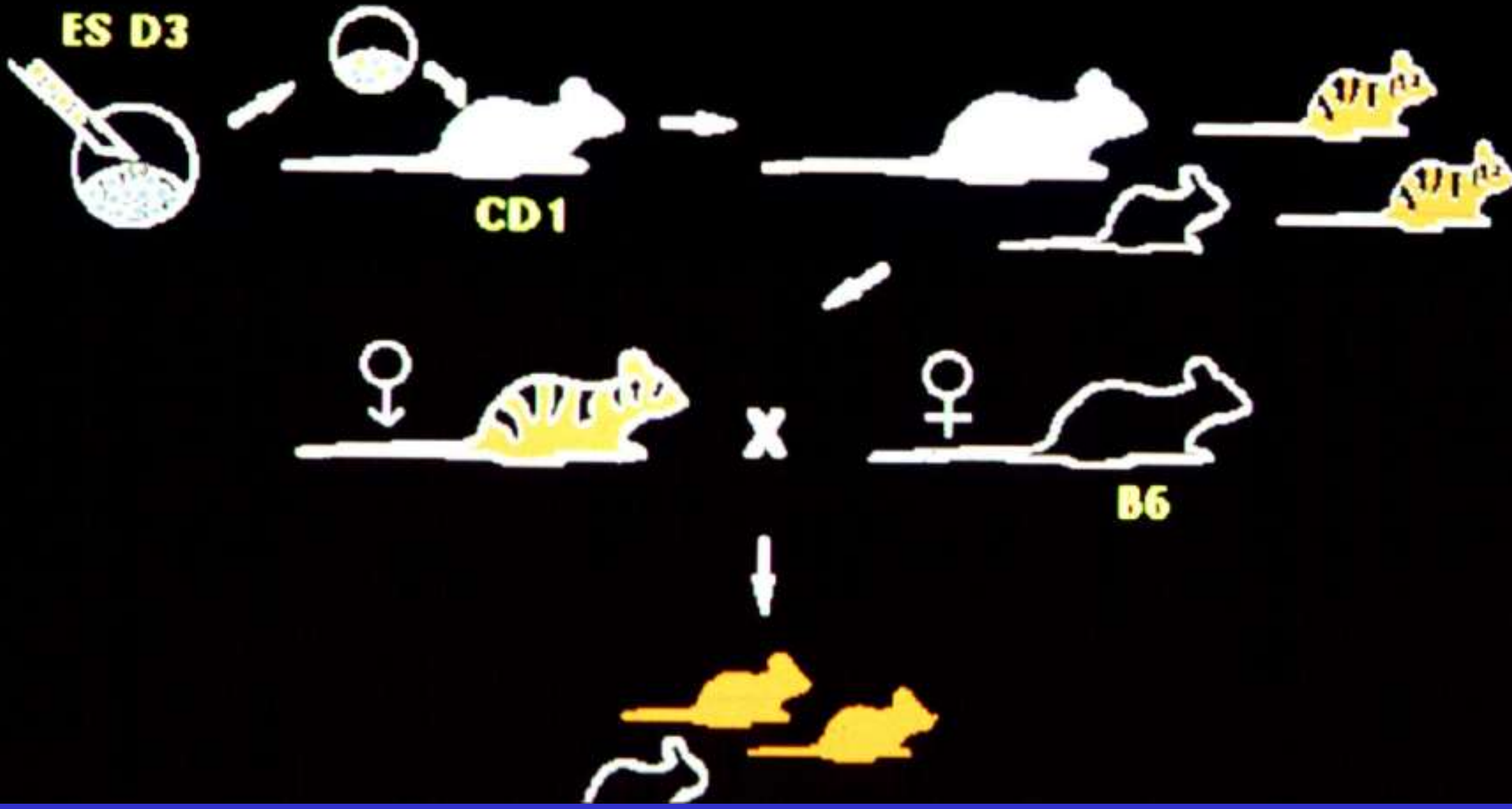


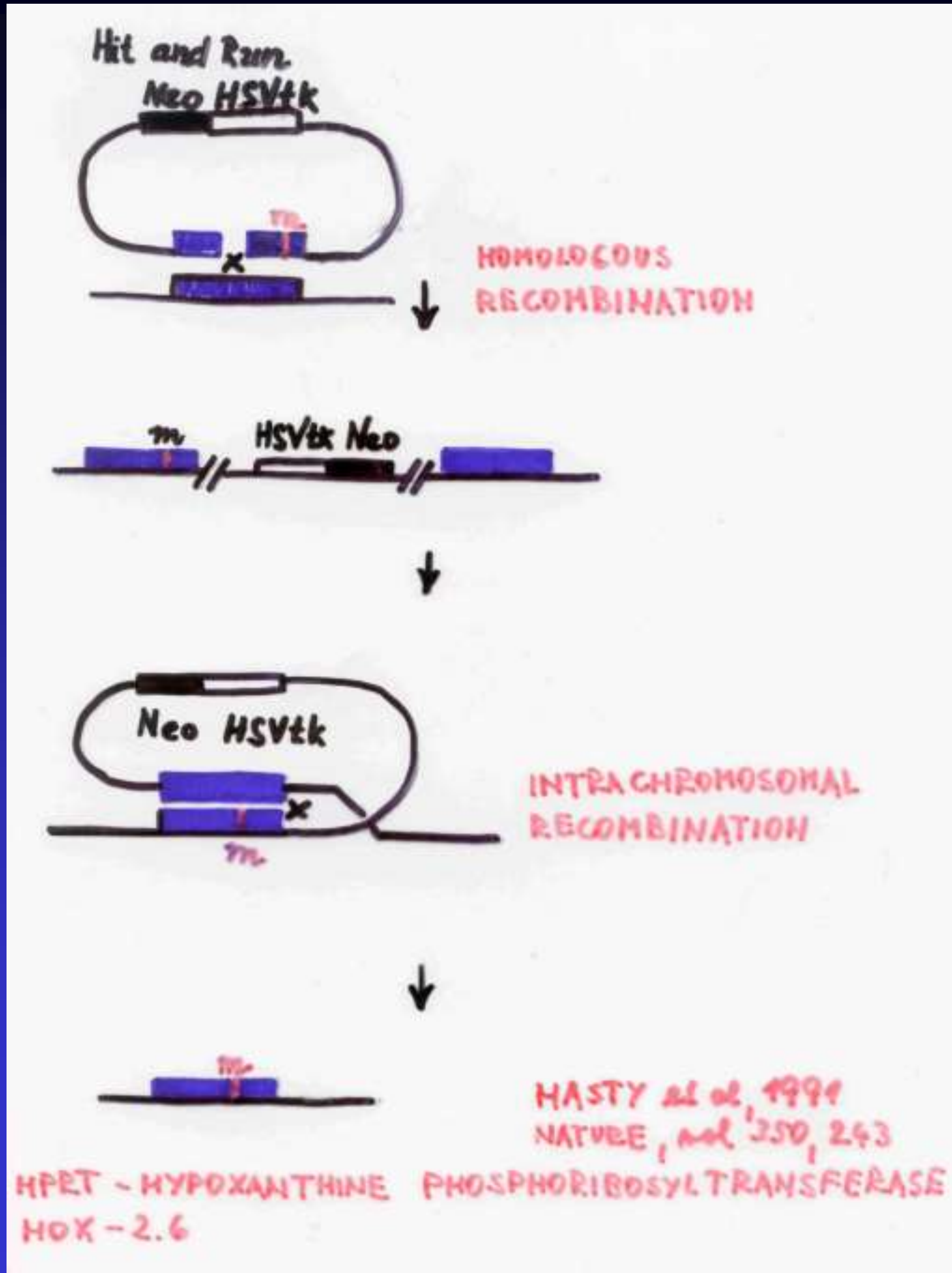
Southern blot analysis of ES E14 cell clones

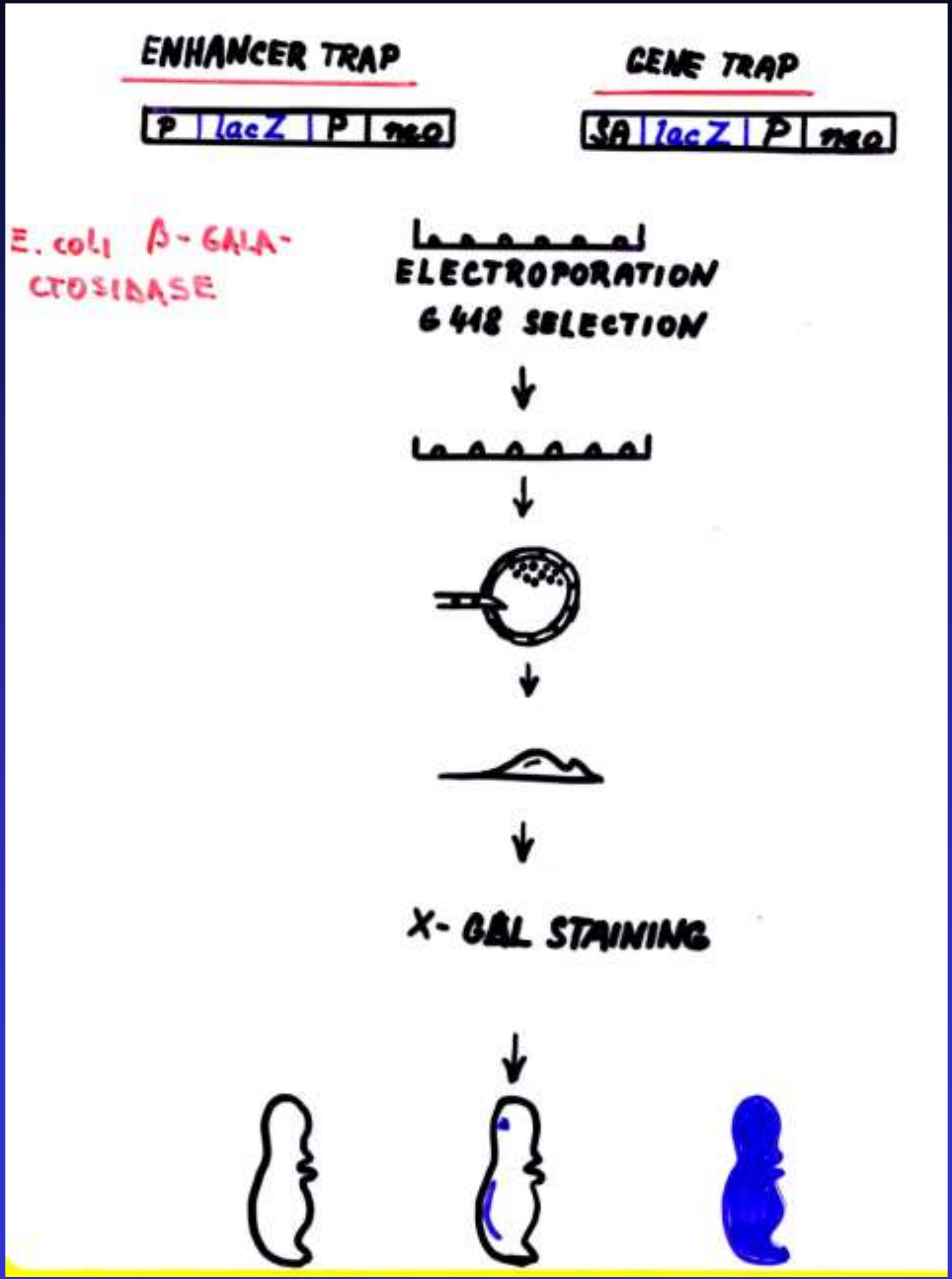




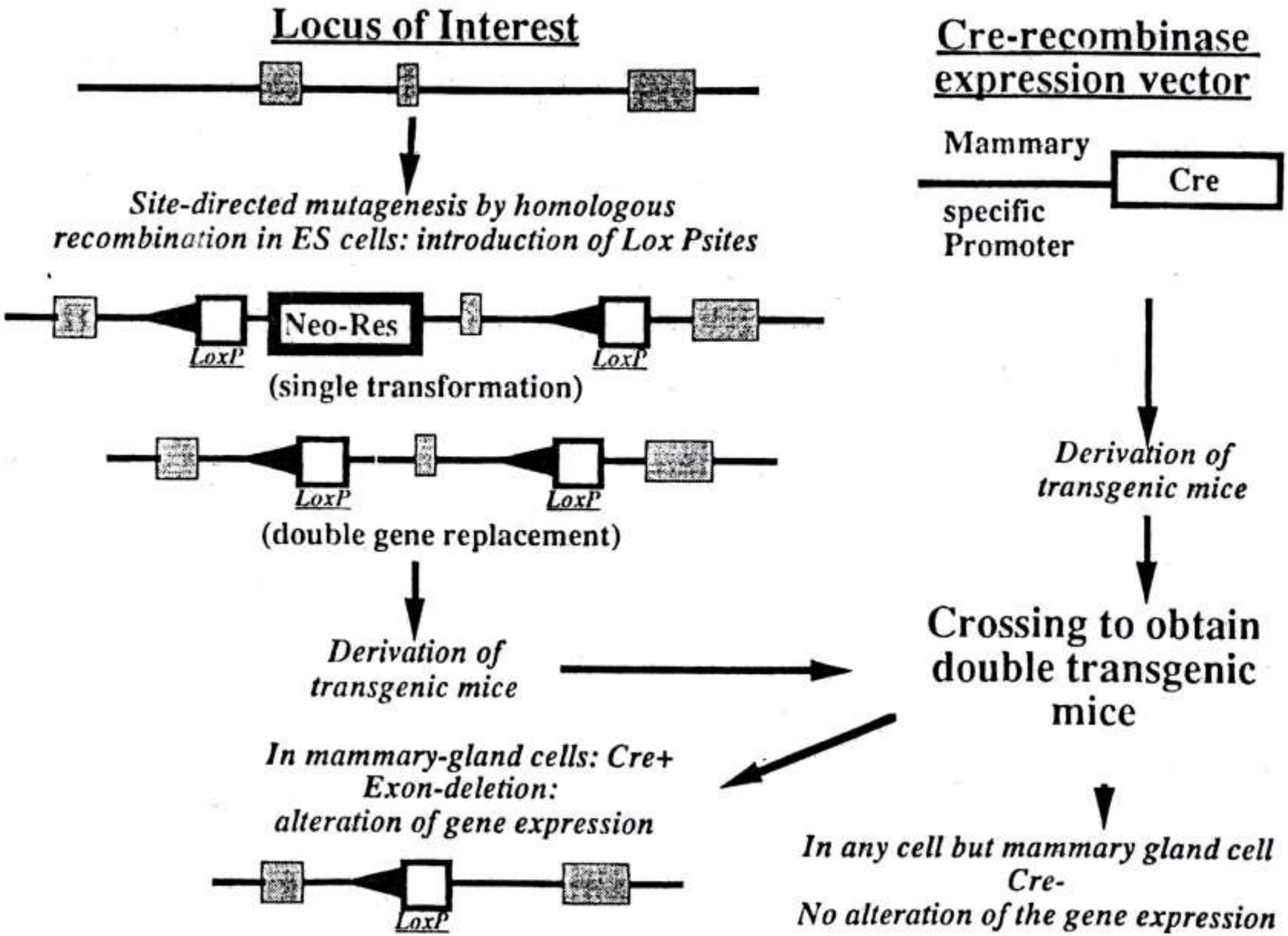
Generation of mouse germ-line chimeras




Hit and Run
Neo HSVtk
m
HOMOLOGOUS
RECOMBINATION
m
HSVtk Neo
Neo HSVtk
INTRACHROMOSOMAL
RECOMBINATION
m
m
HASTY et al, 1991
NATURE, vol 350, 243
HPRT - HYPOXANTHINE PHOSPHORIBOSYLTRANSFERASE
HOX-2.6

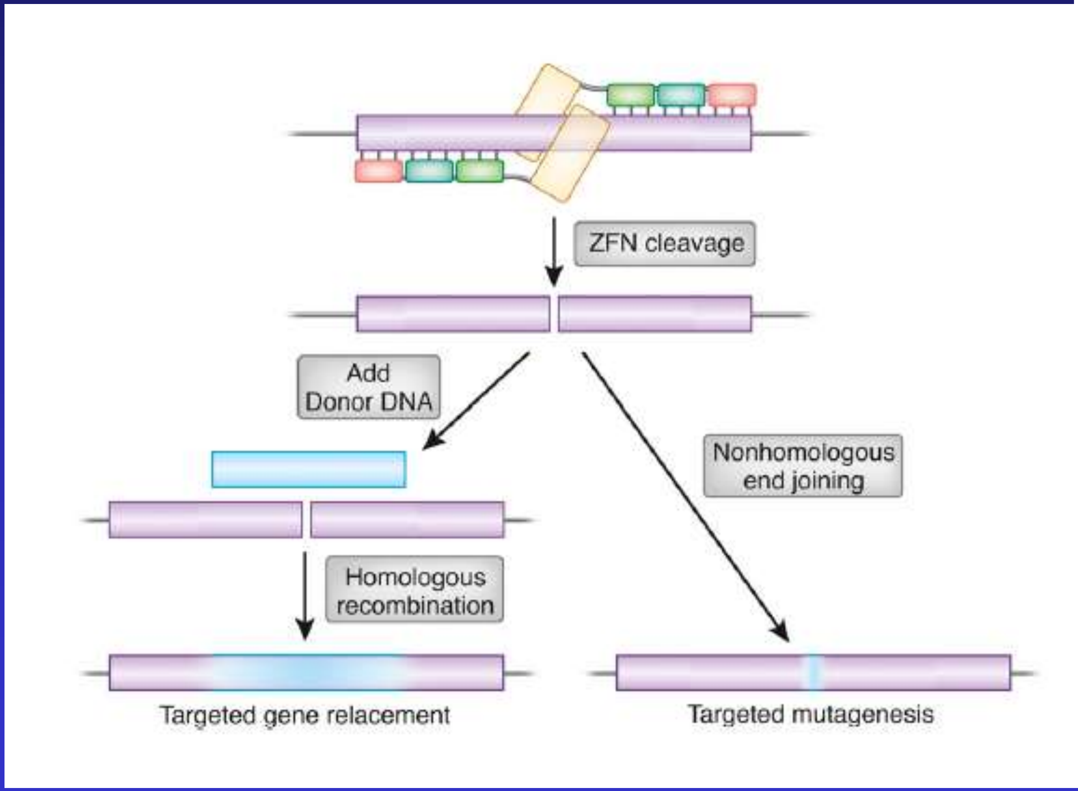


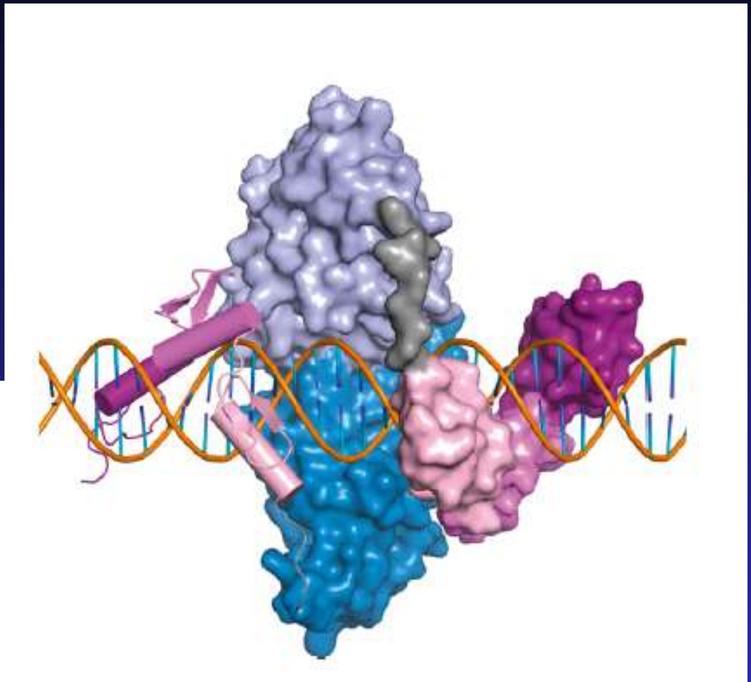
ENHANCER TRAP
P lacZ P neo
GENE TRAP
SA lacZ P neo
E. coli β-GALACTOSIDASE
ELECTROPORATION
G418 SELECTION
X-GAL STAINING


Locus of Interest
Cre-recombinase expression vector
Mammary
Cre
specific
Promoter
Site-directed mutagenesis by homologous recombination in ES cells: introduction of Lox Psites
Neo-Res
LoxP
LoxP
(single transformation)
LoxP
LoxP
(double gene replacement)
Derivation of transgenic mice
Derivation of transgenic mice
Crossing to obtain double transgenic mice
In mammary-gland cells: Cre+
Exon-deletion:
alteration of gene expression
LoxP
In any cell but mammary gland cell
Cre-
No alteration of the gene expression

ZFNs – Zinc finger nukleásy – DNA binding domain + Fok I endonuclease monomer

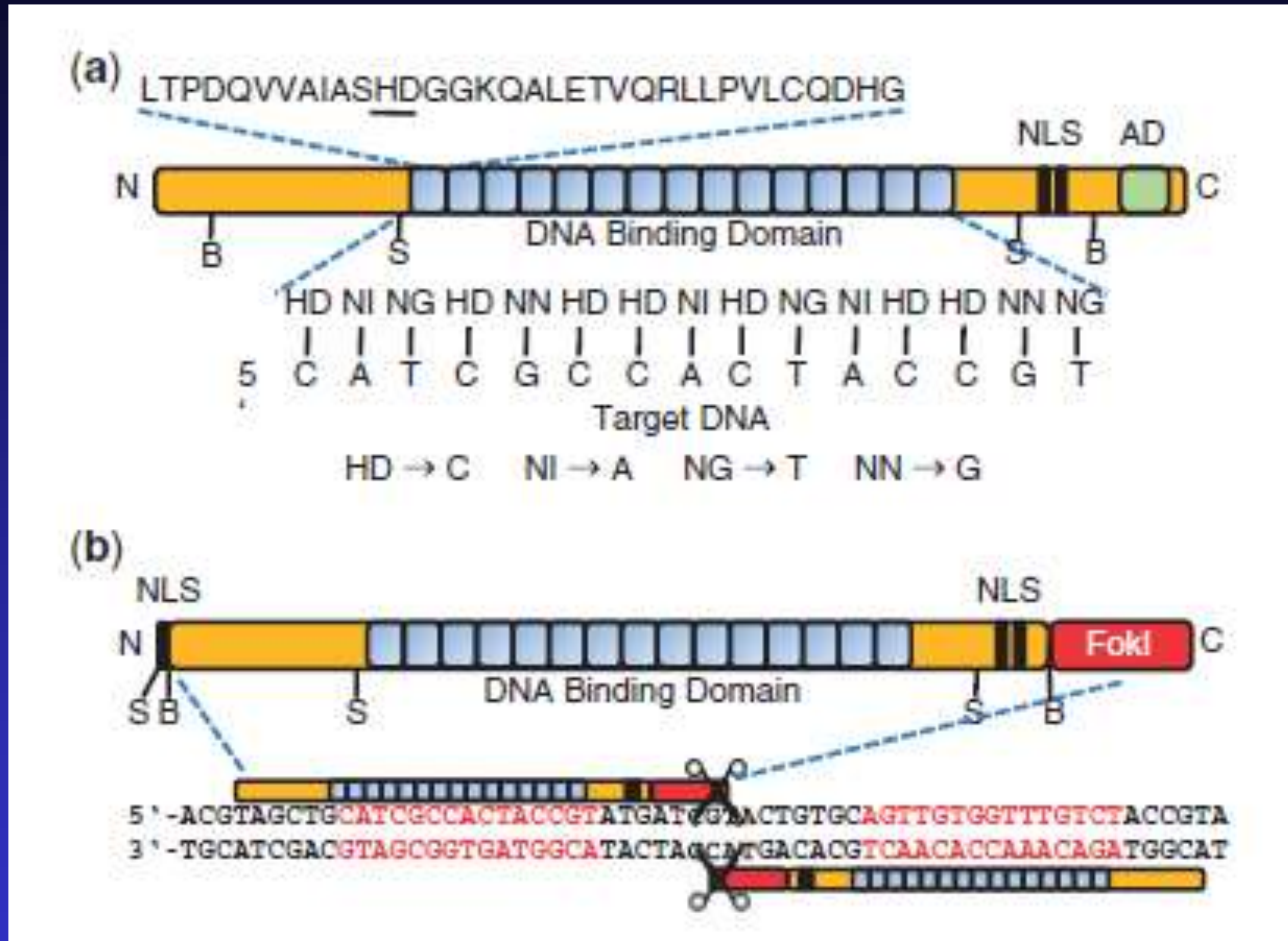
Double-strand break – oprava pomocí non-homologous end-joining, drobné inzerce nebo delece (indels)





Caroll D., Genetics, 188: 773-782, 2011

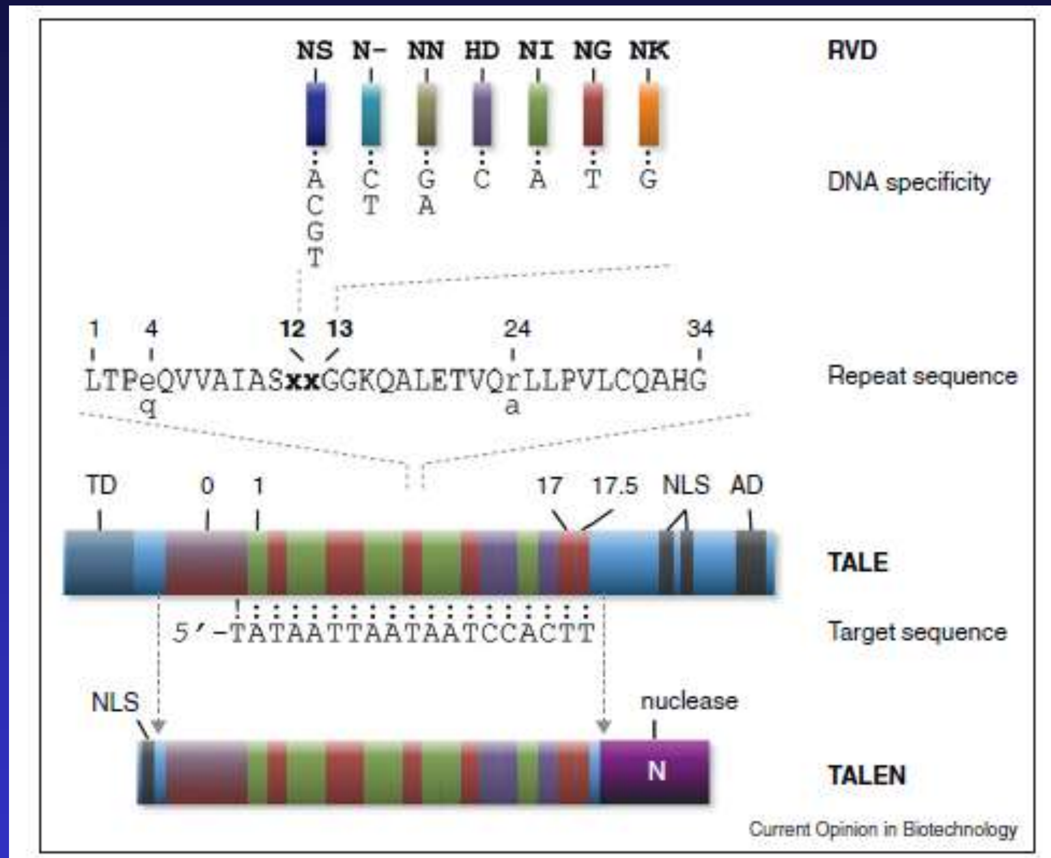
TALENs – transcription aktivátor-like effector nucleases, TALE-based nucleases



TALE – bakteriální proteiny, pathogen Xanthomonas – injikace proteinů do infikovaných rostlinných buněk
Rozpoznání cílové DNA v hostitelském genomu, aktivace exprese genů, nezbytných pro multiplikaci pathogenu

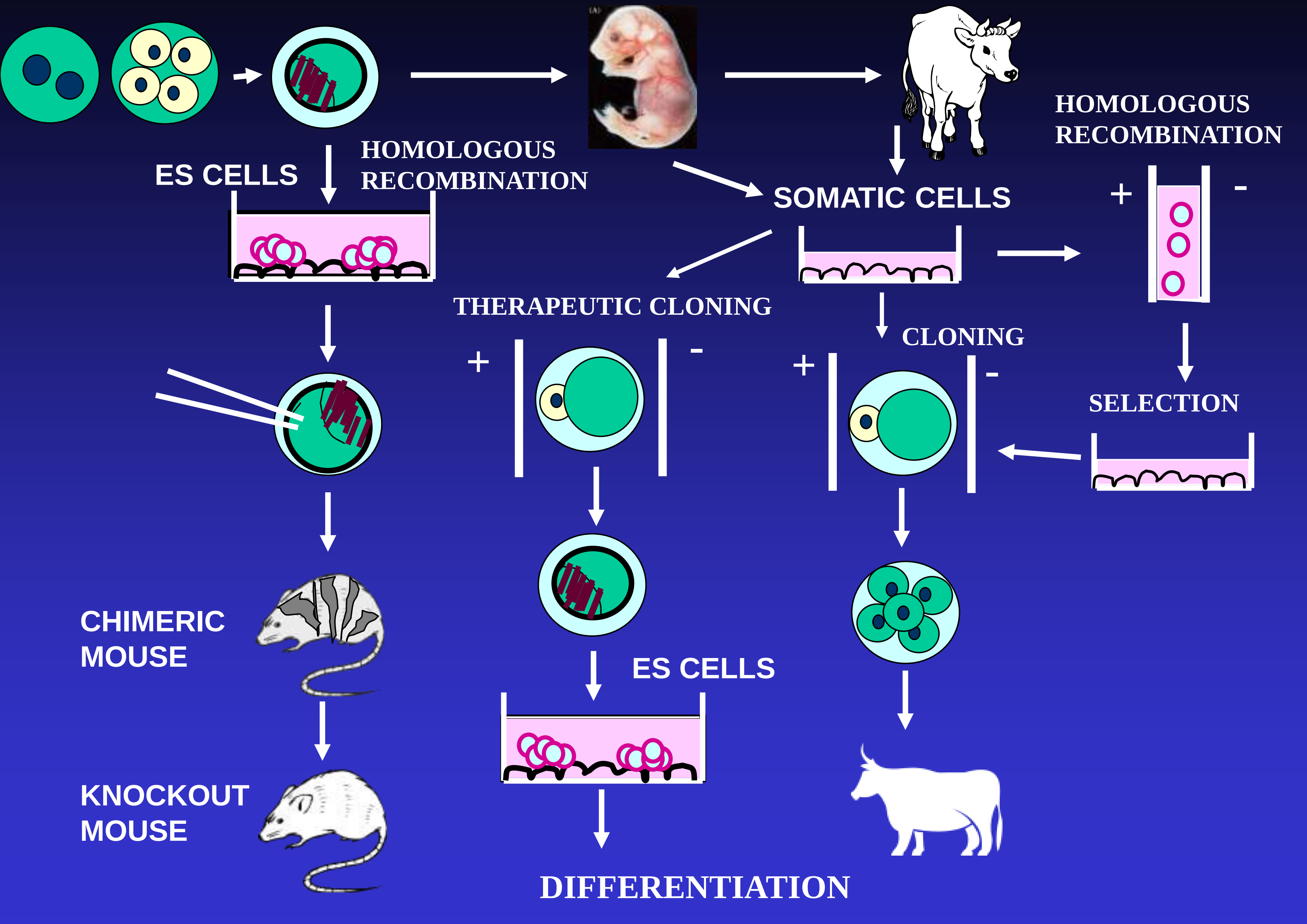
DNA binding domain – skládá se z tandemu 15,5 – 19,5 single repeats, každý se skládá z 34 vysoce konzervovaných zbytků

Carlson D.F. et al., www.pnas.org/cgi/doi/10.1073/pnas.1211446109

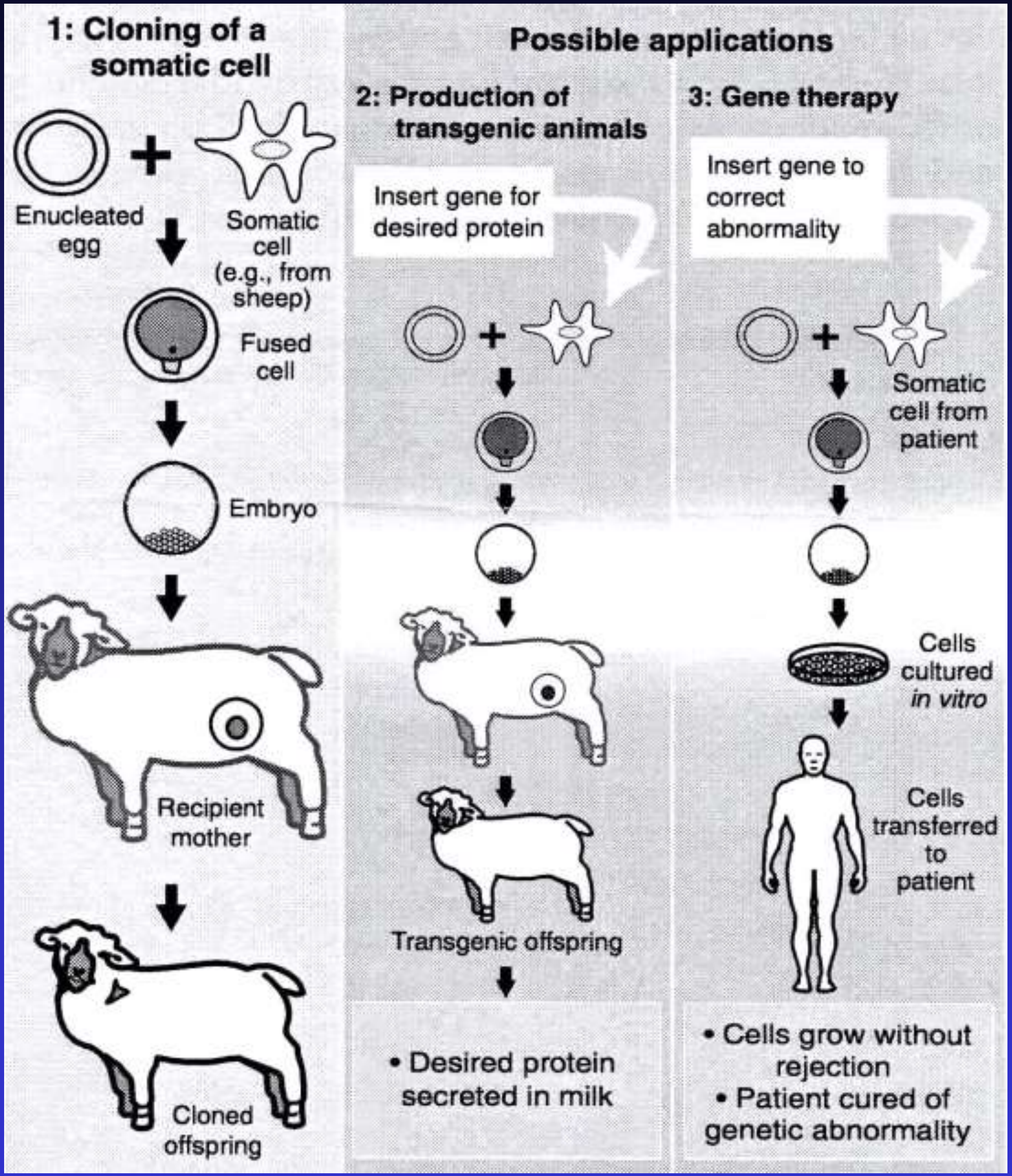


NS N- NN HD NI NG NK
RVD
A C G C A T G
C T A
G
T
DNA specificity
1 4 12 13 24 34
LTPeQVVAIASxxGGKQALETVQrLLPVLCQAHG
q a
Repeat sequence
TD 0 1 17 17.5 NLS AD
TALE
5′-TATAATTAATAATCCACTT
Target sequence
NLS
nuclease
N
TALEN
Current Opinion in Biotechnology

PRE-IMPLANTATION EMBRYO



CLONING - POTENTIAL BENEFITS



Trounson, A. ; MJA 167:568-569 ; 1997