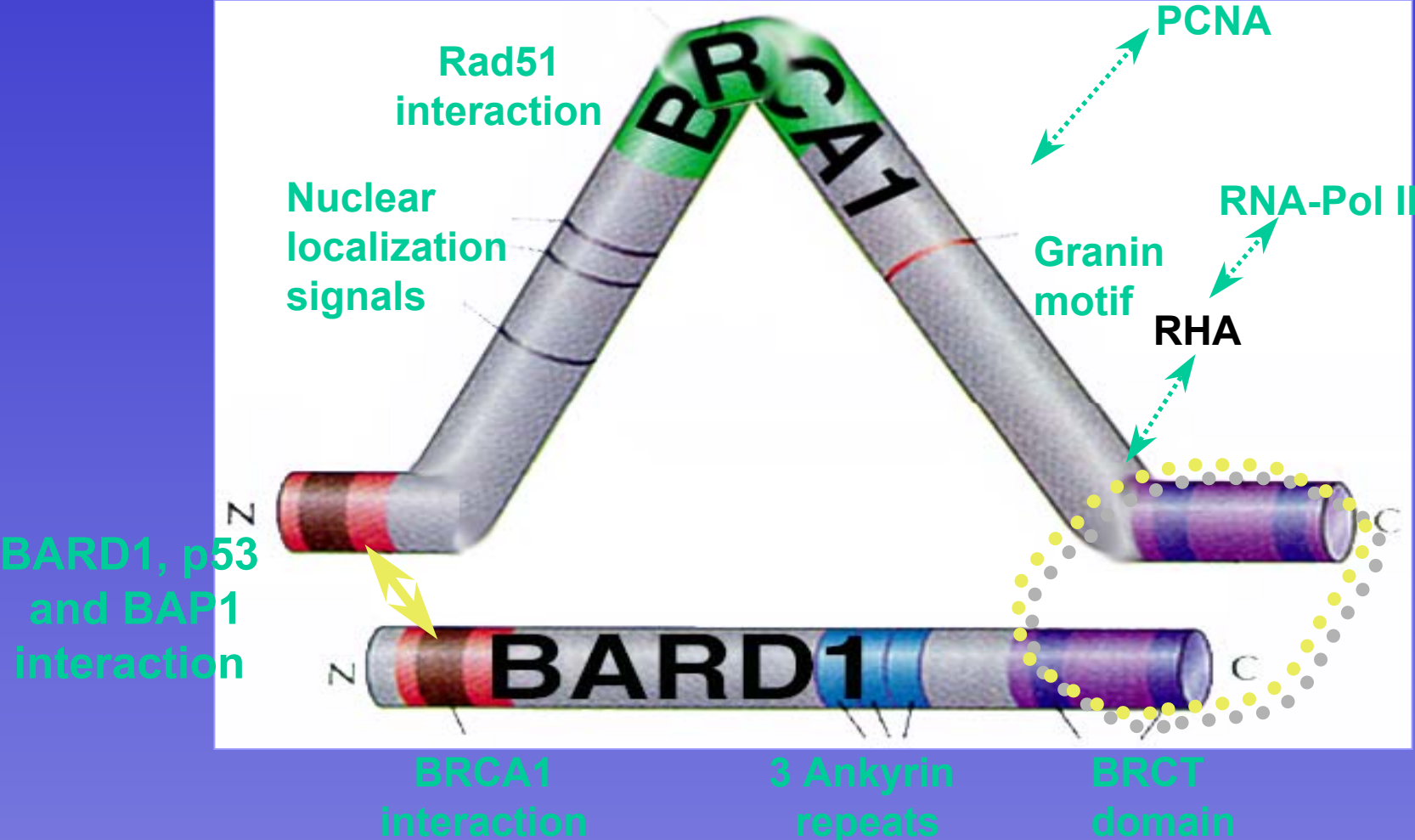


The role of BARD1 and its spliced variant in Spermatogenesis

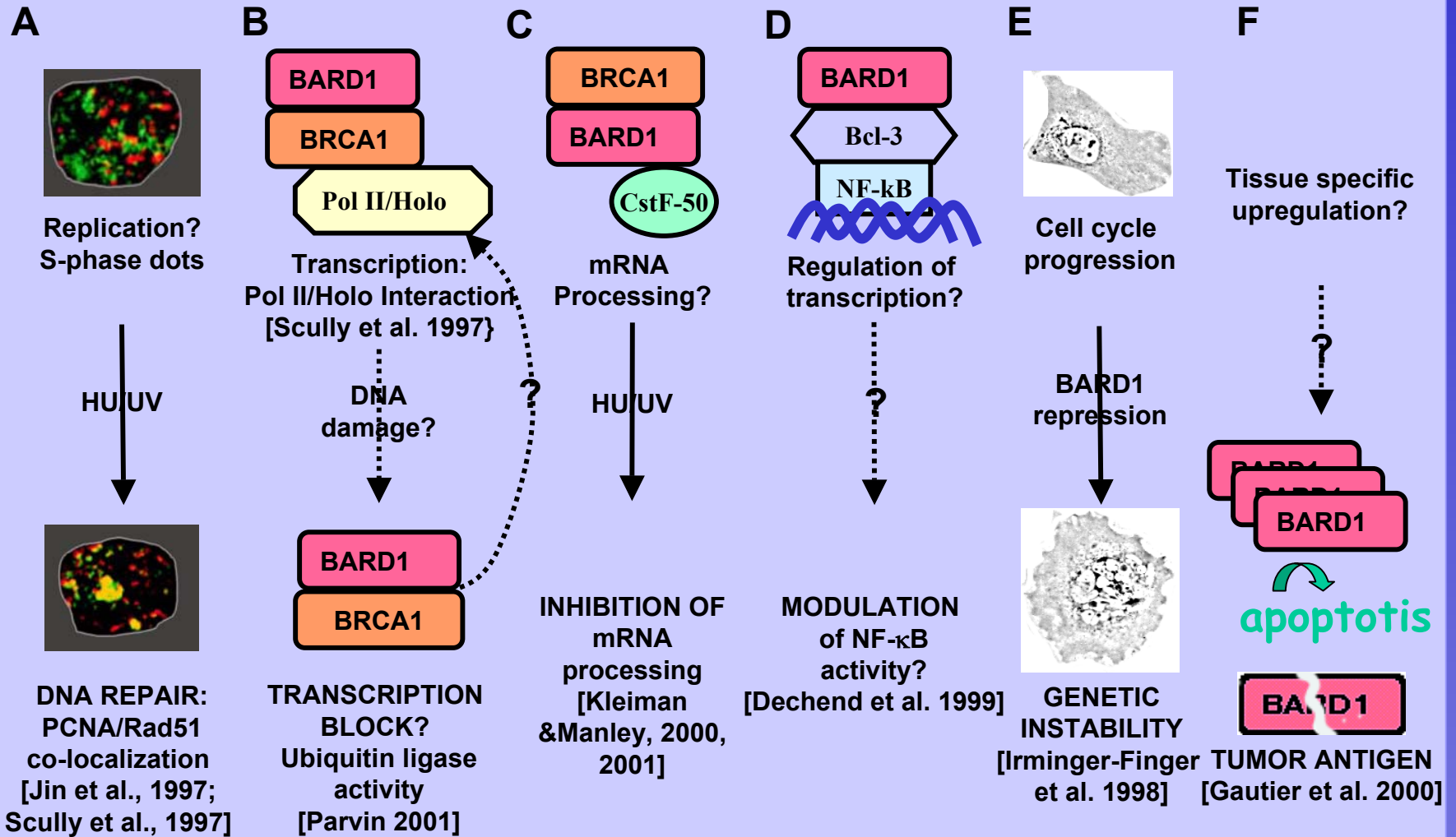
Anis Feki

**Biology of Aging Laboratory
University Hospital of Geneva
Switzerland**

Structure of BARD1 and BRCA1



Cellular Functions of BARD1

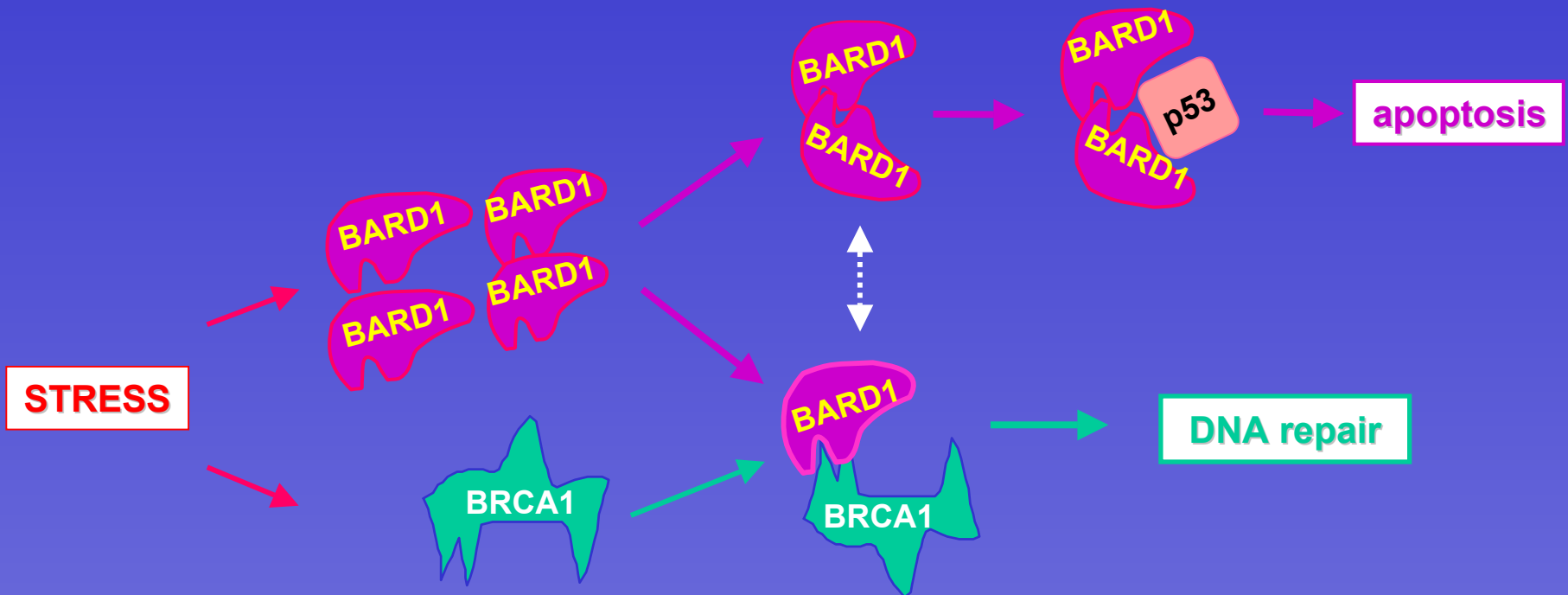


TUMOR SUPPRESSOR FUNCTIONS *Irminger-Finger, IJBCB, 2002*

Introduction to the BARD1 bimodal function

- **BRCA1 function in apoptosis** (Harkin et al)
- **BARD1 function may depend on its association with BRCA1 via the ring finger** (WU et al)
- **BARD1 may induce apoptosis independently of its associated with BRCA1**
 - Only BARD1 but not BRCA1 increased after stroke
 - Co-transfection of BRCA1 did not enhance BARD1 induced apoptosis
 - BARD1 induced apoptosis in BRCA1 -/- cells.

Bimodal function of BARD1



Irminger-Finger et al, 2001

Did the bimodal function exist in the testis?

- BARD1 and BRCA1 are highly expressed in testis.
- Meiosis, DNA repair and apoptosis are frequent events in this tissue.
- Apoptosis is frequent in testis from healthy and fertile individuals of many species.

Spermatogenesis

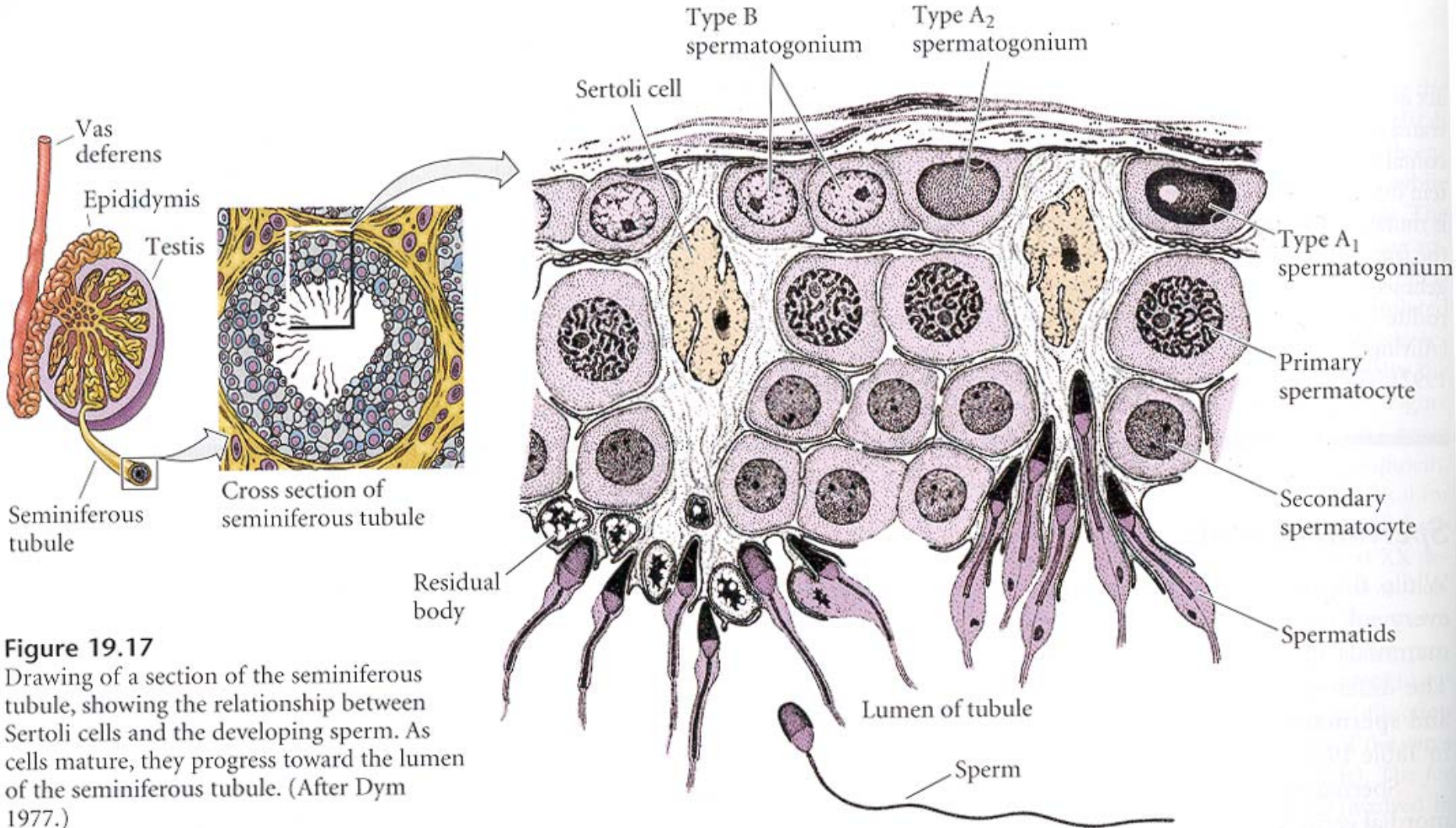
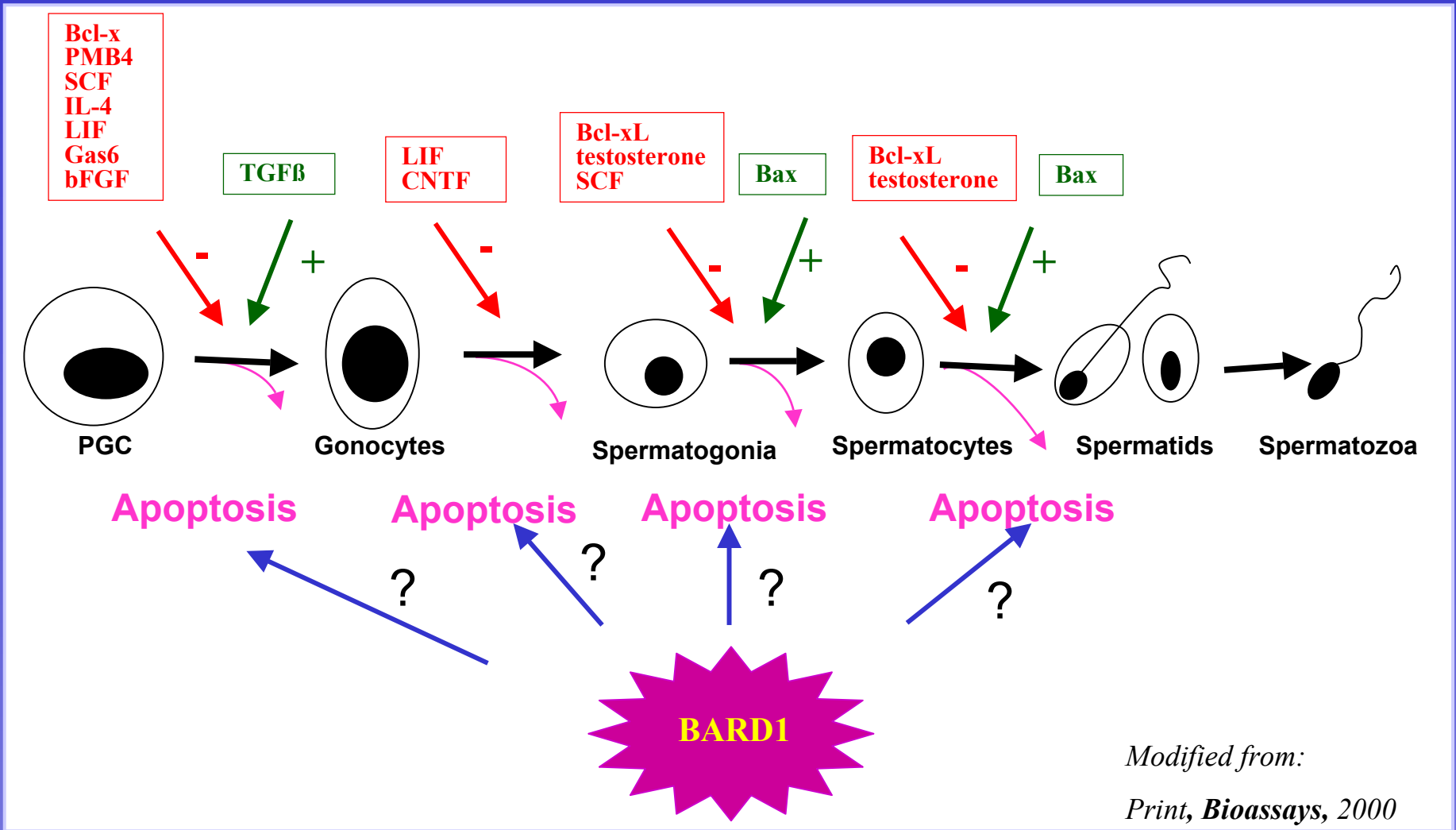
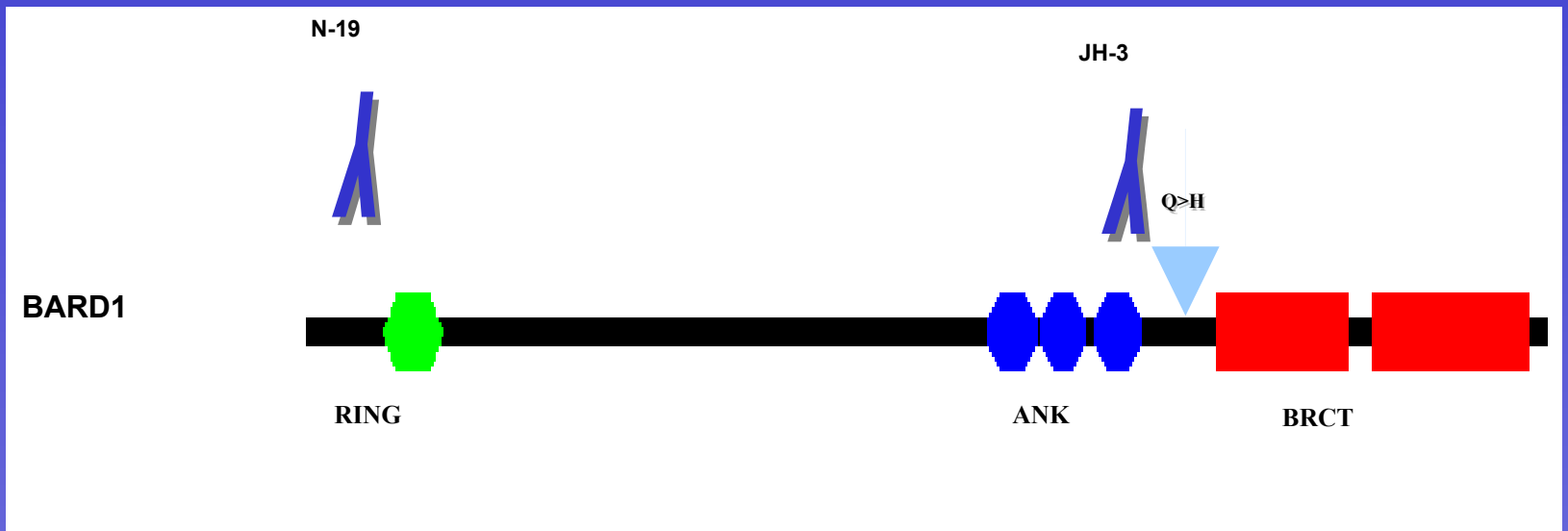


Figure 19.17
Drawing of a section of the seminiferous tubule, showing the relationship between Sertoli cells and the developing sperm. As cells mature, they progress toward the lumen of the seminiferous tubule. (After Dym 1977.)

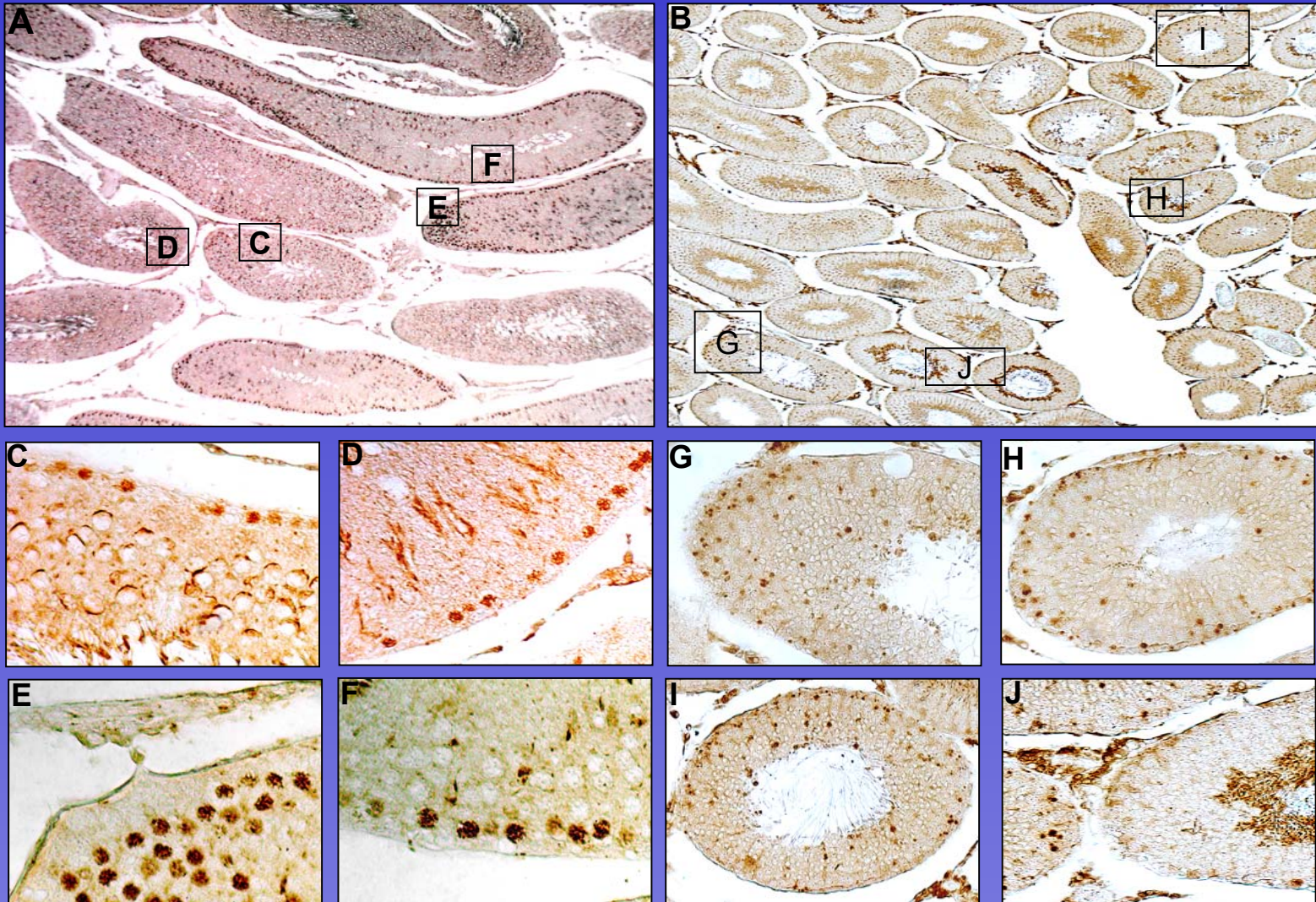
Apoptosis during spermatogenesis



Modified from:
Print, *Bioassays*, 2000

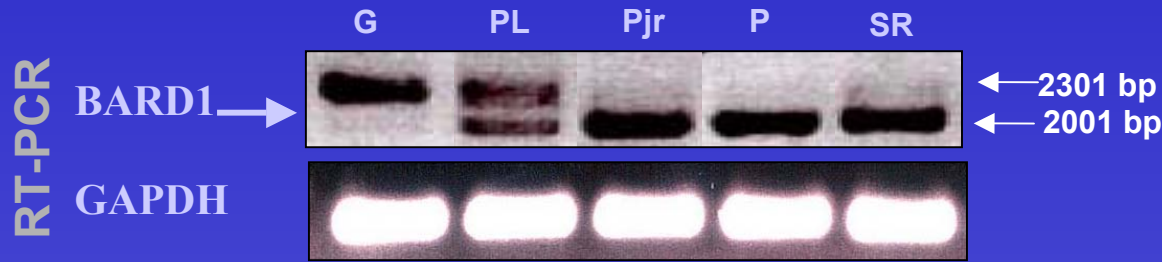


Expression of BARD1 in spermatogenesis



BARD1 N-19

BARD1 JH3



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Human atgccggataatcggcagccgaggaaccggcagccgagggatccgctccgggaacagagcctcgttccgcgcccgccatggaaccggatggtcgcgggtgctgggcccacagtcgcgcgcgctcgaccgcctggagaag
Mouse -----atgccacgcgcggccgcgaggggtctgctctgggaaccagcctgctcccggtgcccgccatggagccggctaccgacggggtttGggcccacagccgcgcggcgttgcccgcctggagaag
Rat -----atgccacgcgcggccgcgaggggtctgctccgggaacaagcctcctcccggtgcccgccatggaaccagctaccgacggggtttgggcccacagccgtgcccgccttgcccgcctggagaag
BETA -----ATGCCACGCCGGCCGCGAGGGTCTGCTCCGGGAACAAGCCTCCTCCCGTGCCCGCCATGGAACCAGCTACCACGGGGCTTTGGGCCACAGCCGTGCGGGCGCTTGCCCGTCTGGAGAAG

Human ctgctgcgctgctcgcgttgtactaacattctgagagagcctgtgtgttaggaggatgtgagcacatcttctgtagtaattgtgtaagtgactgcattggaactggatgtccagtgtgttacaccccgccctggata
Mouse ctgctgcgctgctcccgcgtgtgctaataatctgaaggagcccggtgtgcttaggaggatgtgagcacatcttctgtagtggttgataaagtgactgtgttggtccaggatgcccaagtgtgttacacccagcctggatc
Rat ttgttgcgctgctcccgcgtgtgctaataatctgagggagcccggtgtgcttaggaggatgagagcacatcttctgtagtggttgataaagcactgtgttggtcaggatgcccaagtgtgttacacccagcctggatc
BETA TTGTTGGGCTGCTCCCGCT- [REDACTED]

Human caagacttgaagataaatagacaactggacagcatgattcaactttgtagtaagcttcgaaatttgctacatgacaatgagctgtcagatttgaagaagataaacctaggaaaagttgtttaatgatgcaggaaac
Mouse ctagacctcaagataaacgacaattggacagcatgatccagcttctagtaagctccaaaatttgctccatgacaataa----agattcaaaagacaacacatctagggaagtttatttggtgatgcaggaaag
Rat ctagacctcaagataaacagacagttggacagcatgatccaactttataagtaagcttcaaaaatttactacatgacaataaaggttcagattcaaaagacgacacatctagggaagtttatttggtgatgcaggaaag
BETA [REDACTED]-GATTCAAAAGACGACACATCTAGGGCAAGTTATTGGTGATGCAGAAAAG

Human aagaagaattcaatataaatgtggttttagcctcgaagtaagaagtcagatatgttgtgagtaagcttcagtgcacaaccagcctgcaataaaaaaagatgcaagtgtcagcaagactca---tattatg-gctg
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Rat aagaagaattcagtaaaaaatgtggttttagtcctcgaagtaagaataatagatgttgtgtaataaagtttcagtacaaaccagcctcaaaaggcaaaaggatgacaaagcccaggaagcctcagtggttttattccagc
BETA AAG AAGAATTCAGTAAAAATGTGGTTTAGTCTCGAAGTAAGAAAATTAGATGTGTTGTGAATAAAGTTTCAGTACAAACCAGCCTCAAAAGGCAAAGGATGACAAAGCCAGGAAGCCTCAGTGTTTTATTCCAG

.....
HUMAN tgaaaagaaatcatagaggagagactttgctccatattgcttctattaaggggcagacataaccttctgttgaataccttttacaaaatggaagtgatccaaatgttaaagaccatgctggatggacaccattgcatgaag
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RAT caggaaaagaaatcacagaggagagacgttactgacattgctctatataagggtgatataaccttctgttgaataccttctgcaaaaatggaaacgacccaaatgttaaagaccatgctggatggacaccgttgcataga
BETA CCAGGAAAAGAAATCACAGAGGAGAGACGTTACTGCACATTGCCTCTATA [REDACTED] GCATGA

HUMAN --cttgcaatcatgggcacctgaaggt
MOUSE agcctgcagtcacatgggcacctgaaggt
RAT agcctgcagtcacatgggcacctgaaggt
BETA AGCCTGCAGTCATGGGCACCTGAAGAT

```

P: Pachytene spermatocyte (3 months old), SR: Round spermatid (3 months old),

Pjr: Pachytene spermatocyte (23 days old), PL: Preleptotene spermatocyte (23 days old), G: Spermatogonia (9 days)

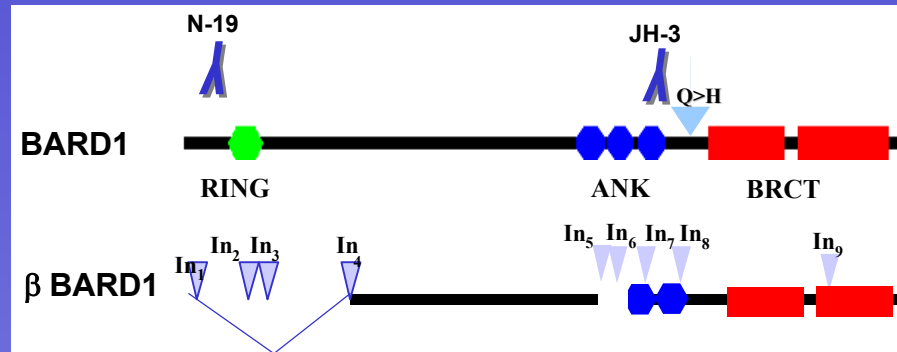
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Human_BARD1 MPDNRQPRNRQPRIIRSGNEFRSAPAMEPDGRGAWAHSRAALDRLEKLLRCSRCTNIIILREPVLGGCEHIFCSNCVSDICIGTGPCVCYTPAWIQDLKINRQLDSMIQLCSKLRNLLHDNELSDLKEDKPRKSLFNDAGNKNSIKMWFSPRSKKVRYVVKASVQTQPAIKKDAQAQ
Mouse_BARD1 ----MPR-RPPRVCSGNQPAPVPAMEPATDGLWAHSRAALARLEKLLRCSRANILKEPVCLGGCEHIFCSGCISDCVSGGCPVCTPAWILDLDKINRQLDSMIQLS SKLQNLHLDNKK--DSKDNTSRASLFGDAERKNSIKMWFSPRSKKVRYVVKVSVQTQPKAKDDKAQE
Rat_BARD1 ----MPR-RPPRVCSGNKPPVPAMEPATDGLWAHSRAALARLEKLLRCSRANILREPVLGGCEHIFCSGCISDCVSGGCPVCHTPAWILDLDKINRQLDSMIQLY SKLQNLHLDNKGSDSKDDTSRASLFGDAERKNSIKMWFSPRSKKIRCVVNVKSVQTQPKAKDDKAQE
BARD1_beta -----MWFSPRSKKIRCVVNVKSVQTQPKAKDDKAQE

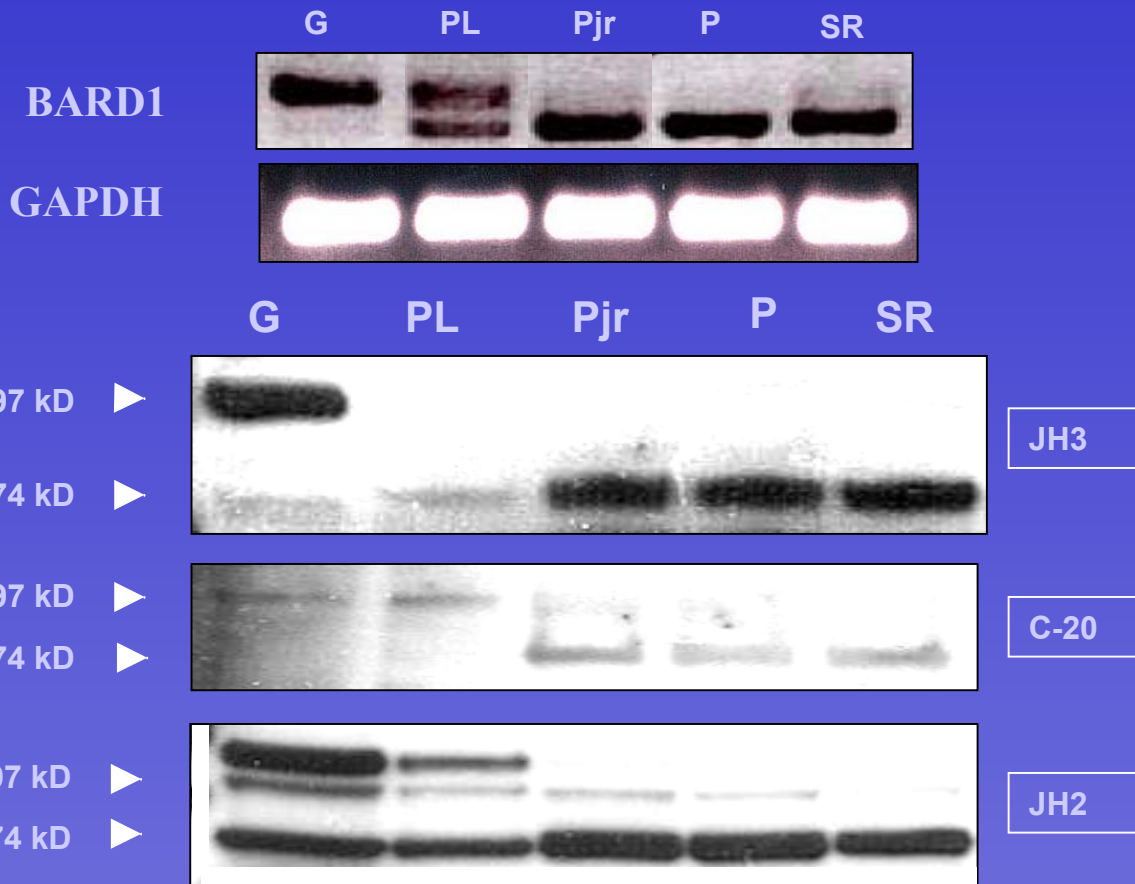
Human_BARD1 DS-YEFVSPPPADVSERAKKASARSQKQKKTAEINQKWNLEAEKEDGEFDSKEESQKLVFSQSPVSISSPQINGEIDLLASGSLTESECFGLTEVSLPLAEQIESPDTKSRNEVVTPEKVCKNYLTSKKSPLLENNGKRGHHRNLSPI SKRCRTS ILSTSGDFVKQTVF
Mouse_BARD1 ASMYEFVSATPPVAVPKSAKTASRTS AKKHPKKSVAKINREENLRPETKDSRFDSKEELKEEKVVSCSQIPVMERPRVNGEIDLLASGSVVEPECSGSLTEVSLPLAEHI VSPDTVSKNEETPEKKVCV----KDLRSGGSNGNRKGCCHRPTTSTSDSCGSI PSTSRGIGEPALL
Rat_BARD1 ASVFEFVSATPPVVVSTRAKTASRTS AKKHPKKSVAKINREGNFRPETRDSRFDSKEKLEKKEKVVVFSQTLVMENSRVNGEIDLLASGSVVEVFGSFAEVS LPLAEHI VSPDTVSKSEEAPEKKVCV----EDRCPVGS DGNPKGCHRPTTSTSKKCGSNVPSASGEI REPTLL
BARD1_beta ASVFEFVSATPPVVVSTRAKTASRTS AKKHPKKSVAKINREGNFRPETRDSRFDSKEKLEKKEKVVVFSQTLVMENSRVNGEIDLLASGSVVEVFGSFAEVS LPLAEHI VSPDTVSKSEEAPEKKVCV----EDRCPVGS DGNPKGCHRPTTSTSKKCGSNVPSASGEI REPTLL

Human_BARD1 SENIPLPECSSPPSCKRCKVGGTSGRKNNSMSEFISLSPGTPPSTLSSSSYRQVMSSPSAMKLLPNM-AVKFNHRGETLLHIASIKGDI PSVEYLLQNGSDPNVKDHAGWTPLEACNHGHLK.....
Mouse_BARD1 AENVVLVDCSSLPSGQLQVDVTL-RRKSNASDDPLSLSPGTPPPLLNSTHRQMMSPSTVKLSSGMPARKFNHRGETLLHIASIKGDI PSVEYLLQNGSDPNVKDHAGWTPLEACSHGHLK.....
Rat_BARD1 AENVVLVDCSSLPSGRLQVDVTL-RRQSNASDDSLSLSPGTPPPLLNNSTHRQMMSPSTVKLSSGIPARKFNHRGETLLHIASIKGDI SSVEYLLQNGSDPNVKDHAGWTPLEACSHGHLK.....
BARD1_beta AENVVLVDCSSLPSGRLQVDVTL-RRQSNASDDSLSLSPGTPPPLLNNSTHRQMMSPSTVKLSSGIPARKFNHRGETLLHIASIK-----HEACSHGHLK.....

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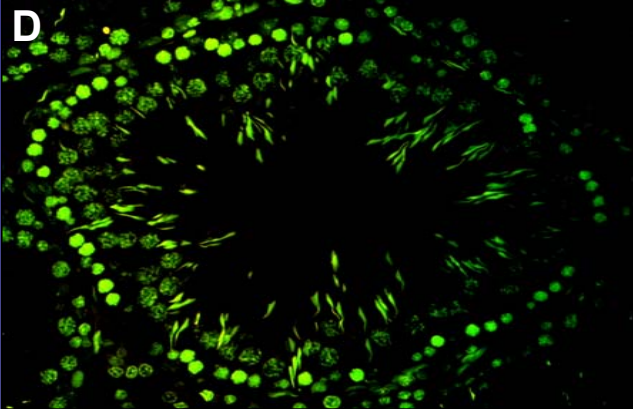
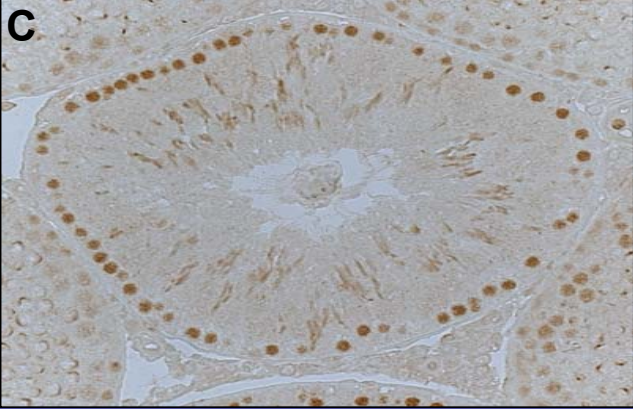
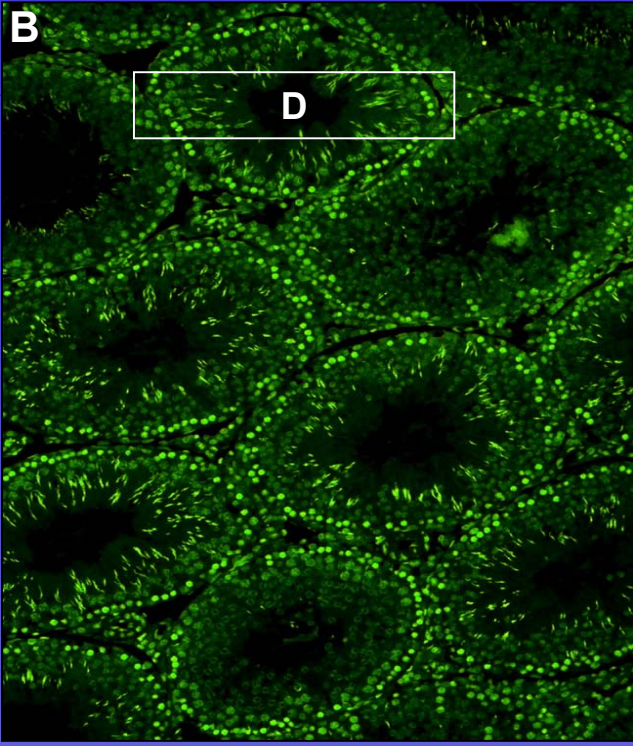
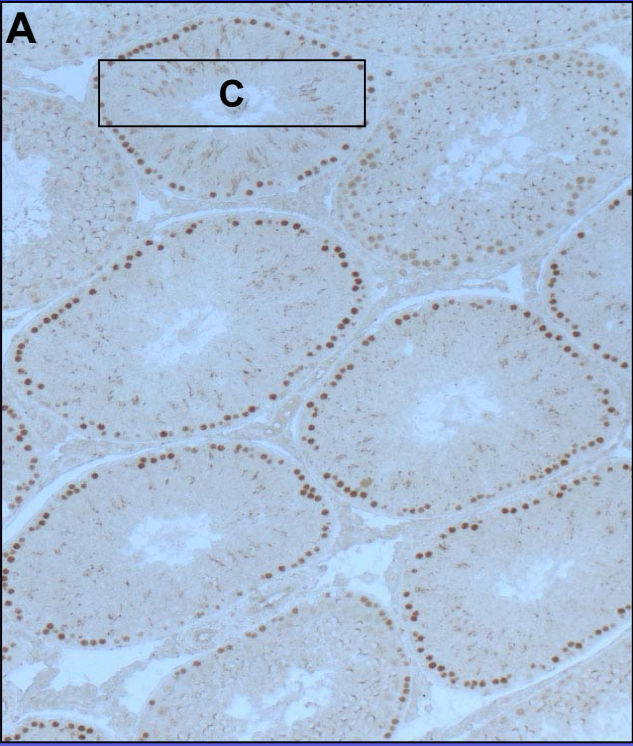
Western blot



P: Pachytene spermatocyte (3 months old), SR: Round spermatid (3 months old),

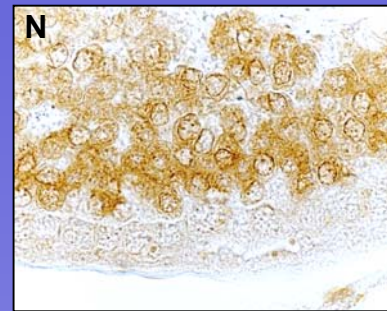
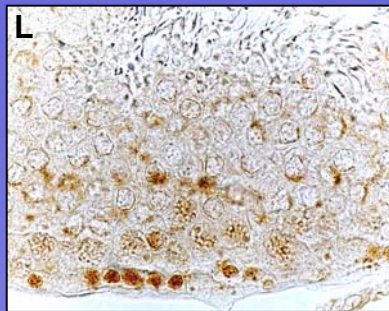
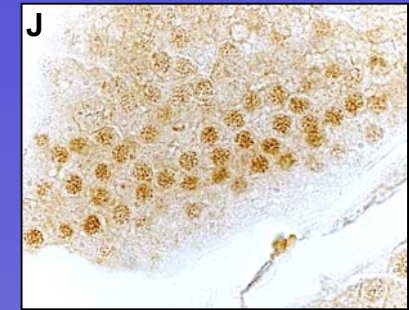
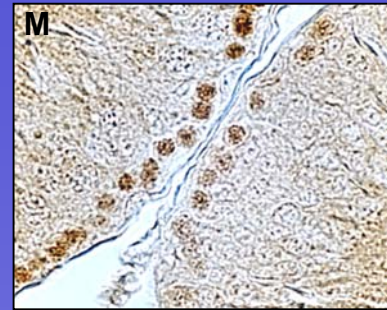
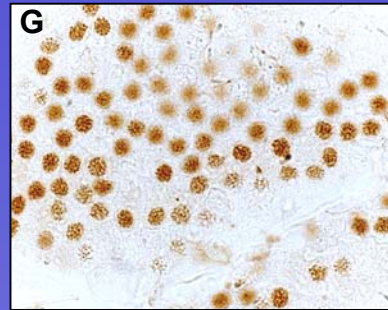
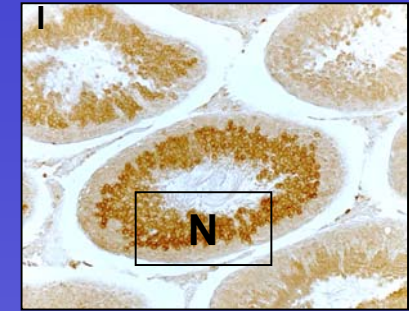
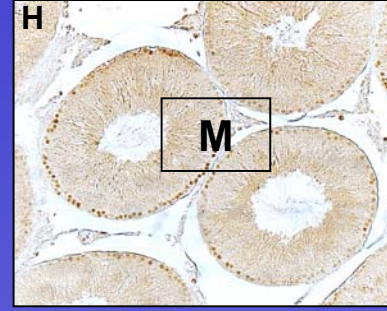
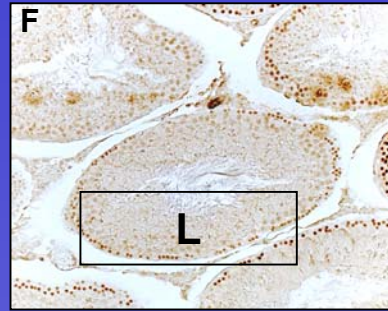
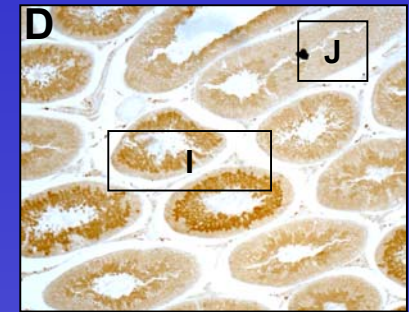
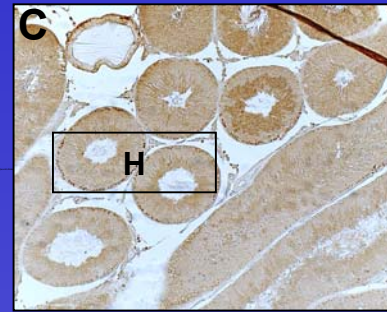
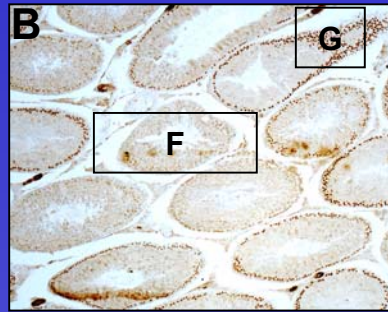
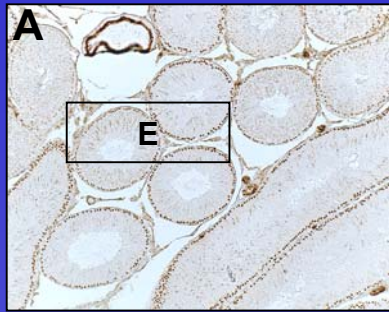
Pjr: Pachytene spermatocyte (23 days old), PL: Preleptotene spermatocyte (23 days old), G: Spermatogonia (9 days)

BARD1 expression is correlated with apoptosis



BARD1 N-19 antibody

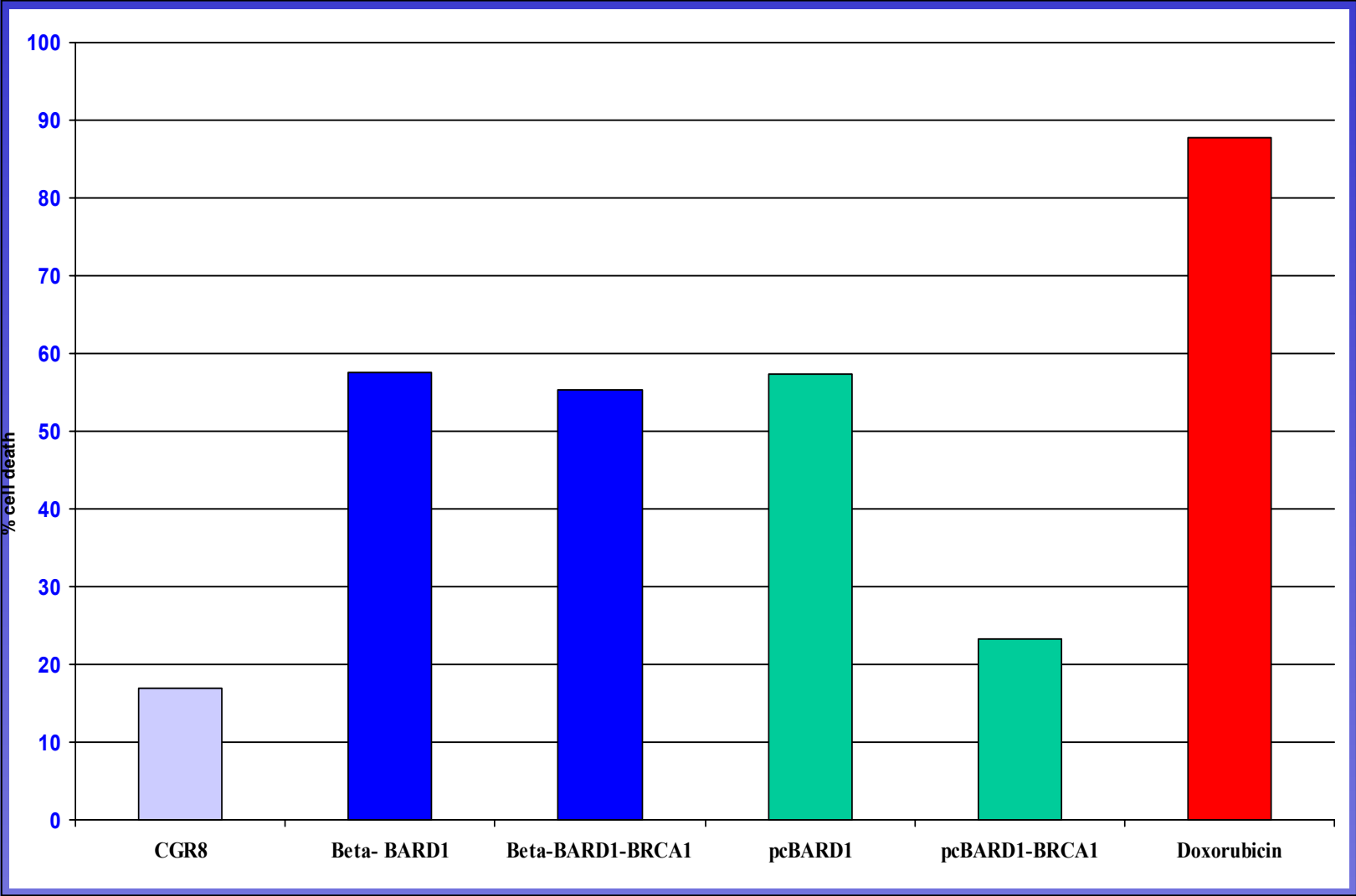
TUNEL



BARD1

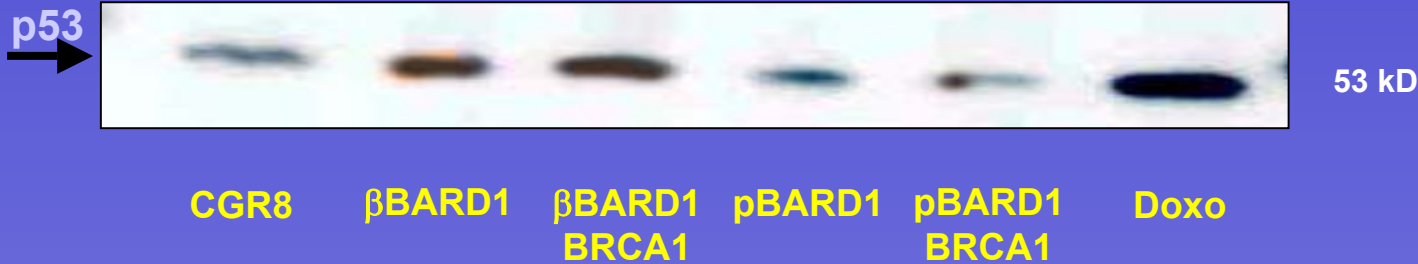
Activated caspase 3

BARD1 isoforms and apoptosis in ES transfected cells



BARD1 isoforms and apoptosis in ES transfected cells

Western blot

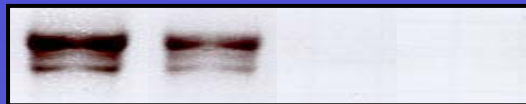


Preleptotene spermatogonia

Sertoli conditioned media

F-/T- F-/T+ F+/T- F+/T+

BARD1



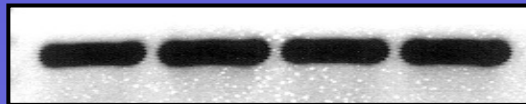
BRCA1



P53



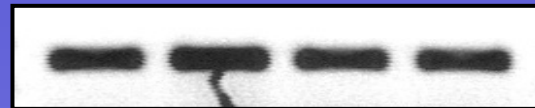
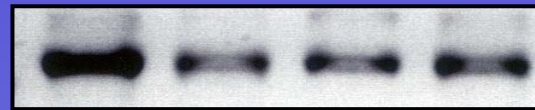
β Tubulin



Round spermatid

Sertoli conditioned media

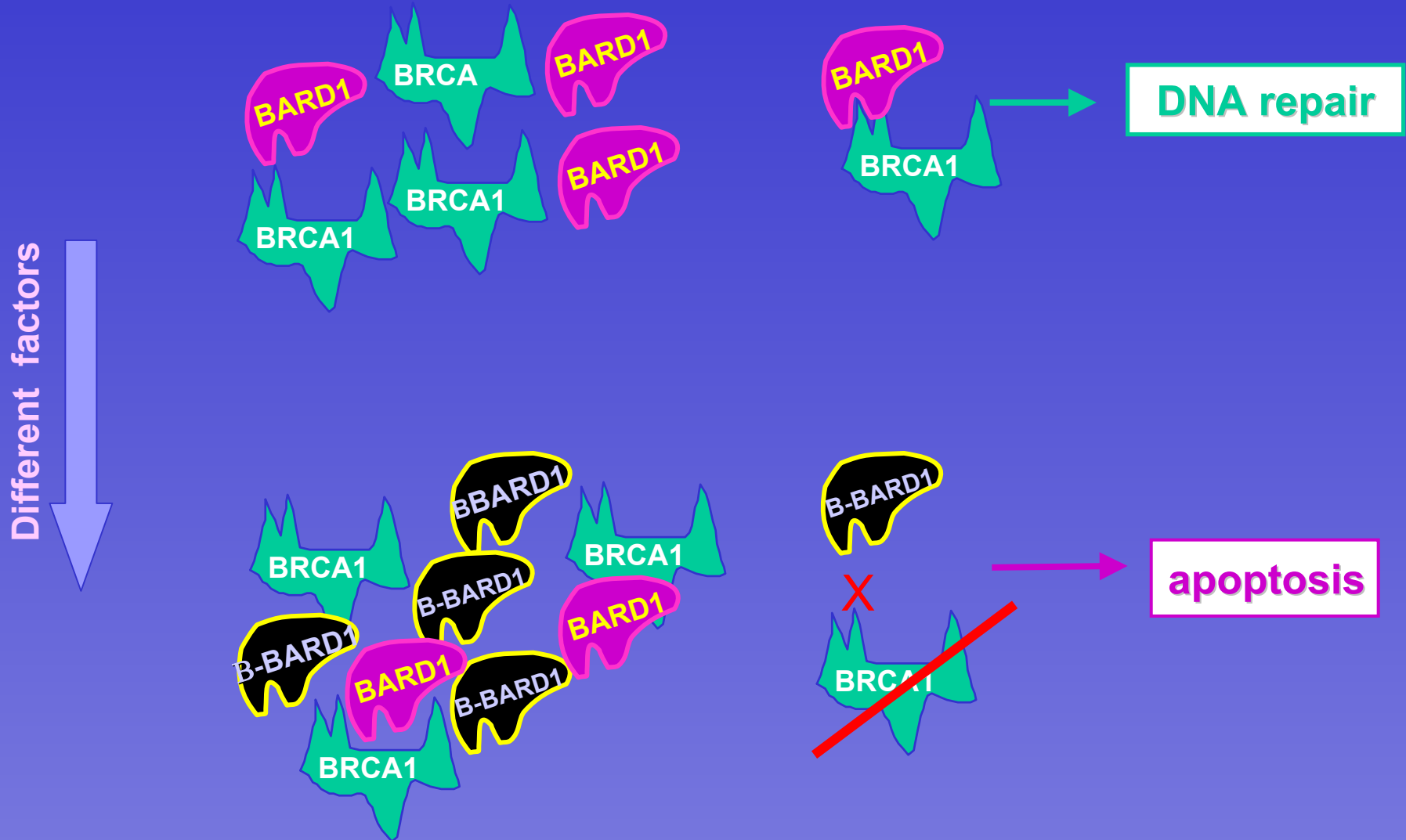
F-/T- F-/T+ F+/T- F+/T+



Concluding remarks

- ➔ Two isoforms of BARD1 were isolated in the testis
- ➔ Both isoforms can induce apoptosis
- ➔ β BARD1 does not interact with BRCA1
- ➔ BARD1 in spermatogenesis may be an example of a bimodal function

What could happen in Spermatogenesis?



Collaborators

- ***Biology of Aging***

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- Charles Edward Jefford
- Mamadu Hadi
- Aurélie Caillon
- Cartier Leatitia
- *Karl Heinz Krause*

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- ***INSERM***

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- J. Harb, Nantes