

**Involvement of retinoid X receptor α and replication factor C
in sea urchin embryonic development**

Sejung Maeng

School of Medicine and Age-Related and Brain diseases Research Center,
Spinal Cord injury Research Laboratory, Kyung Hee University

Nuclear receptor ; Retinoid X receptor

❖ Nuclear receptor (NR)

- N-terminal **A/B region** (a **ligand-independent** activation function-1 (AF-1))
- Central **C region** containing a **DNA-binding domain**
- C-terminal **E region** (a ligand-binding domain and a **ligand-dependent** AF-2)



❖ Retinoid X receptor (RXR)

- The three RXR isotypes (α , β and γ)
- Form heterodimers with many other family members
- Central position in the nuclear receptor superfamily
- Respond to specific for the **9-*cis* retinoic acid**
- Regulating physiological processes essential for **embryonic development as well as for cell growth, differentiation and death**

(Bonnie L. et.al., 1993)

(Nahoum V. et.al., 2007)

(Alvarez R. et. al., 2004)



Vitamin A ; Retinoic acid (RA)

❖ **Vitamin A** is indispensable throughout postnatal development and adult life for growth, survival, reproduction, vision, as well as for the homeostasis of numerous tissues.

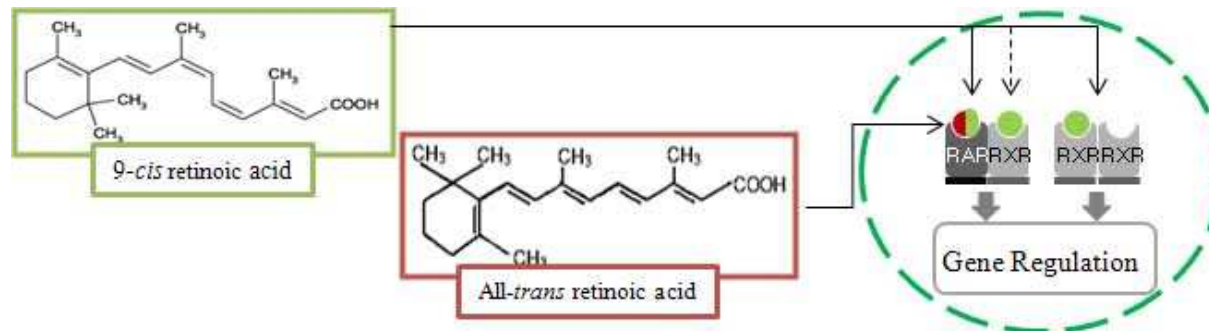
❖ The bioactive retinoids *all-trans* and *9-cis* RA are ligands that bind and activate cognate retinoid receptors, and these receptors, in turn, function as transcription factors that regulate the expression of target genes. (Mary M. et al., 2005)

❖ Retinoic acid receptors (RARs), which binds to *all-trans* and *9-cis* RA with similar affinities, and **retinoid X receptors (RXRs), which bind *9-cis* RA**.

(Shiota G. et al., 2006)

❖ The success of treating APL and estrogen receptor-positive breast cancer patients with retinoids highlighted the fact that cell differentiation therapy is a potent and practical method for the treatment of human cancer.

(Fields et al., J. Cell. Biochem., 2007)

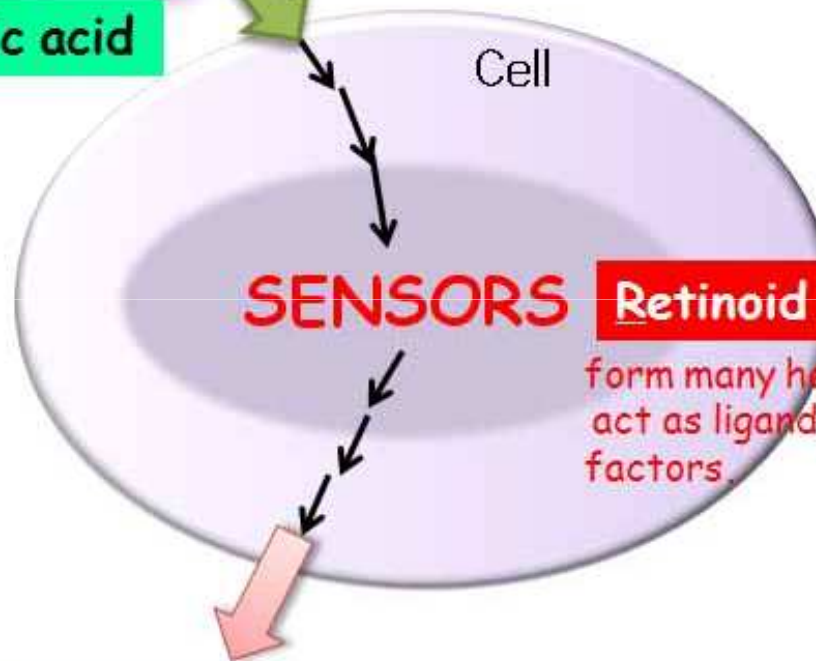
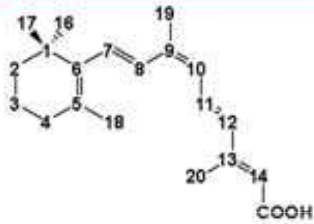


OBJECTIVE

INPUT

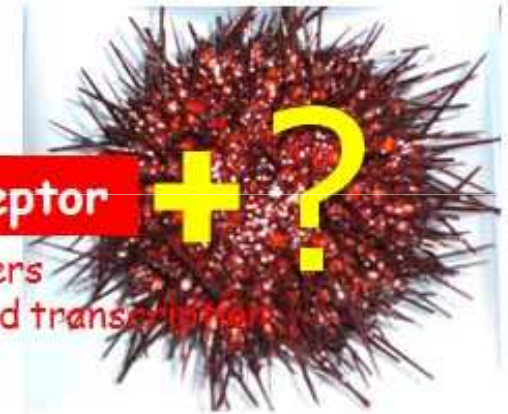
Exogenous/ Endogenous
SIGNALS

9-cis-retinoic acid



SENSORS Retinoid X Receptor

form many heterodimers
act as ligand-activated transcription factors.



OUTPUT

Gene Expression

Cell differentiation,
Proliferation,
Apoptosis

GAL4-based Yeast Two-Hybrid System

- Yeast strain : Y190
- Bait DNA; pGAD10/hRXR-LBD
- Prey DNA; pACT2/*Sp.ovary* cDNA library
- Hormone treatment; 9-*cis* RA

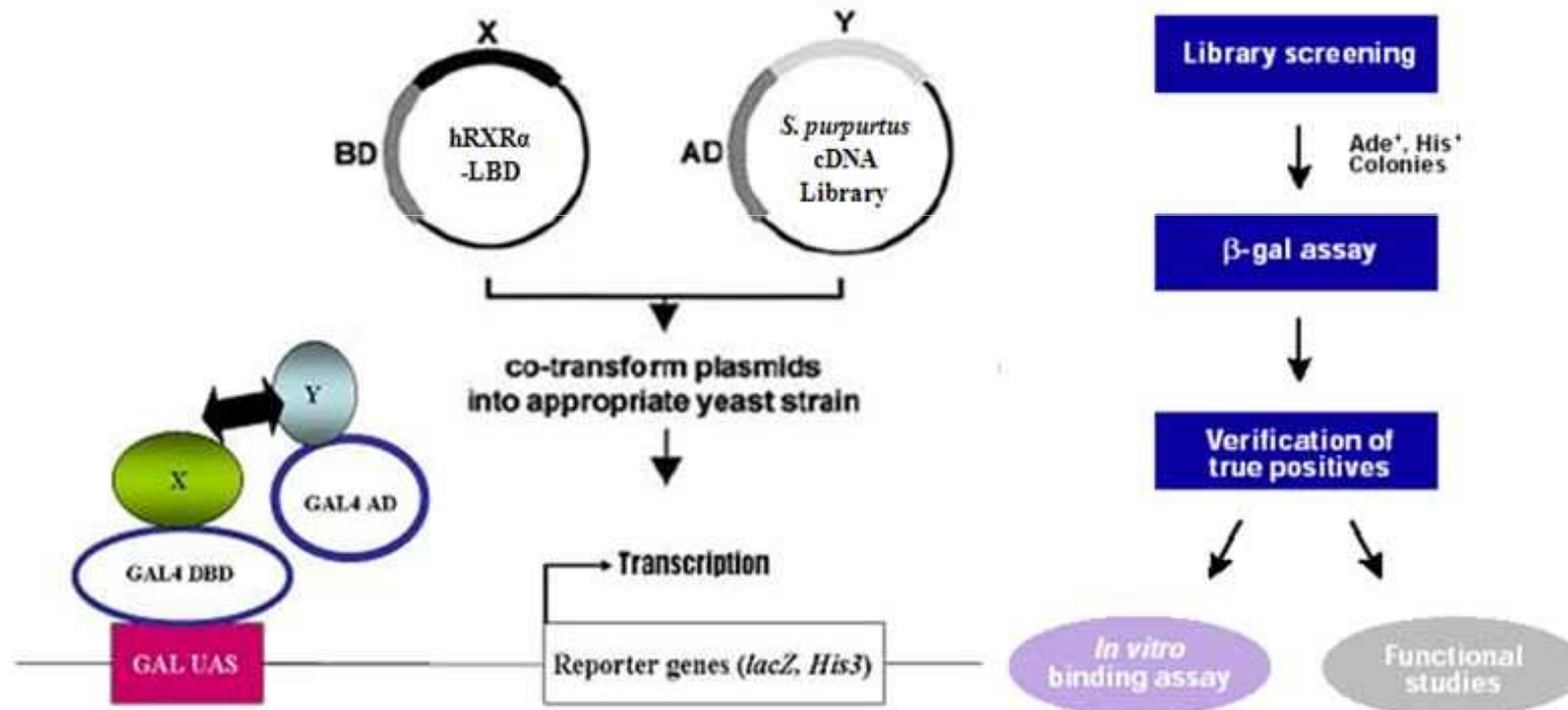


Strongylocentrotus purpuratus

•North American species

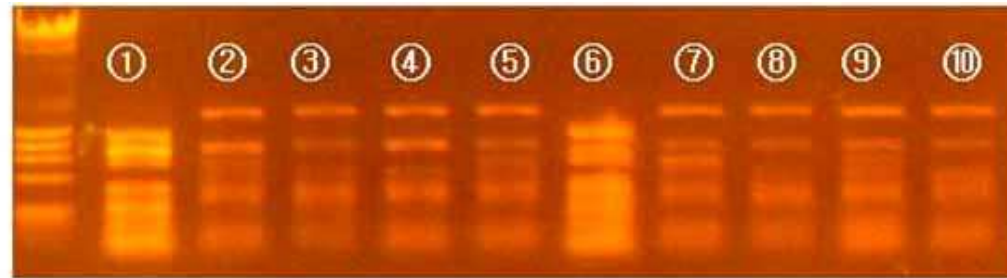
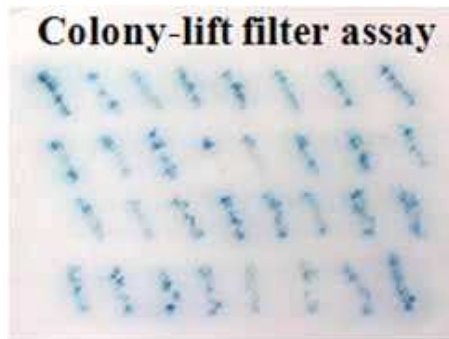
• A target organism for genome analysis by National Human Genome Research Institute of USA.

•Material for screening ; ovary cDNA library from Clontech

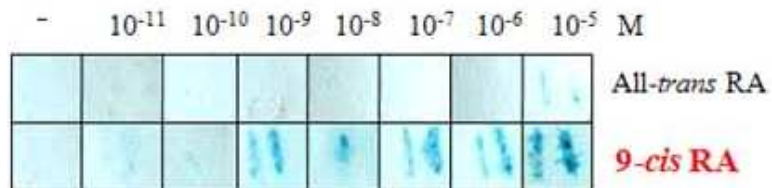


(Field & Song, Nature, 1989)

Screening of Positive Interactions



Representative finger printings of plasmid DNAs using *Hinf* I



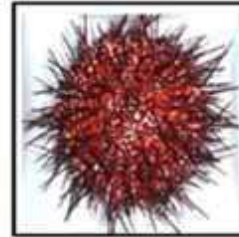
Estradiol -17 β (E2)

Tri-iodothyronine (T3)

Representative results of second colony-lift filter assay after re-transformation

Sequence analysis

	1	60
<i>S. nudus</i>	MSLWVDEKRPSTLSKLDYHKEDQASNLKELVQSGDFPHLMVYGPSSGAGEKTRIMCALKELT	
<i>S. purpuratus</i>	-----A-----	
<i>Homo sapiens</i>	-----Y--C-LGR-----AQ--RN--C-----L-----I-R--	
	61	120
<i>S. nudus</i>	GSGVEKLRIEQQFTTTPSKSKIEITTIASNYHIEVNPSDAGIYDRIVIQDLIRNTAQFQQ	
<i>S. purpuratus</i>	-----E-----A-----	
<i>Homo sapiens</i>	-V-----H-I-----K--S-----L-----N--V--EML-TV--S--	
	121	180
<i>S. nudus</i>	METSAQRDFEVVLTVDRLTKDAQHALRRTMERTTATCRLLI LCCNSTSKVI PAIRSCL	
<i>S. purpuratus</i>	-----	
<i>Homo sapiens</i>	L-N5-----L--K-----MS-----P-----	
	181	240
<i>S. nudus</i>	GVRVAAPSAEITQILQNVCKKBSLMLPSELAKRIAEEKSEFNLRKAL LSCEACKVQQYTF	
<i>S. purpuratus</i>	-----	
<i>Homo sapiens</i>	A--P--ED-CHV-ST-----Q--H-L--C-----L-M--R--	
	241	300
<i>S. nudus</i>	SADQDIPEADWEGFLRETANHI IQQQSPRQLLEVRGRMYELLTHCTPPDVILGHLRELL	
<i>S. purpuratus</i>	-----V--D-----	
<i>Homo sapiens</i>	T--E--T--V-LR--A-VS--T-QR-----L--LL-----EI-M--LS-LL	
	301	356
<i>S. nudus</i>	KNCDGQLNTQVTHQAAFTEHEMQQGNKAIYHLEAFVAKFMSIYKRFLEEGFEAMMF	
<i>S. purpuratus</i>	-----	
<i>Homo sapiens</i>	H-----GE-AQM--Y--L-L-S-----AL--K-M-D-L-G--	



Strongylocentrotus nudus

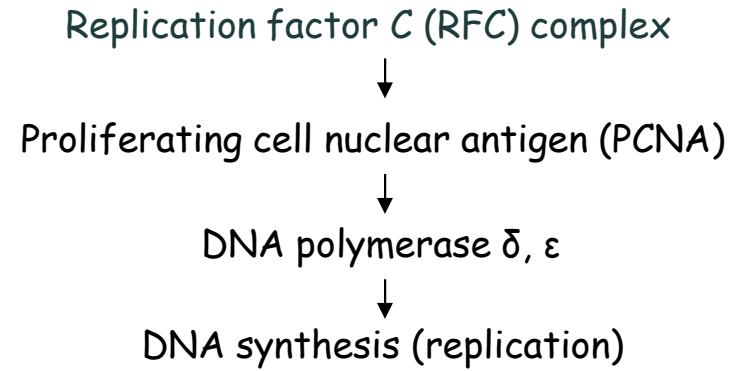
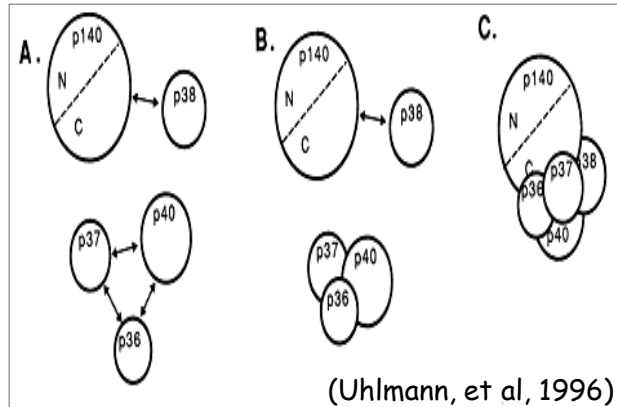
- east Asian species
- Reproductive cycle from June to August
- Material for cloning & functional test

❖ *S. nudus* RFC3 ; *S. purpuratus* 98%,
H. sapiens 76%

❖ LXXLL motif - human (2 ea)
S. nudus (1 ea)

Replication factor C

❖ Identification of RXR-interacting protein ; Replication Factor C 3 (RFC3)

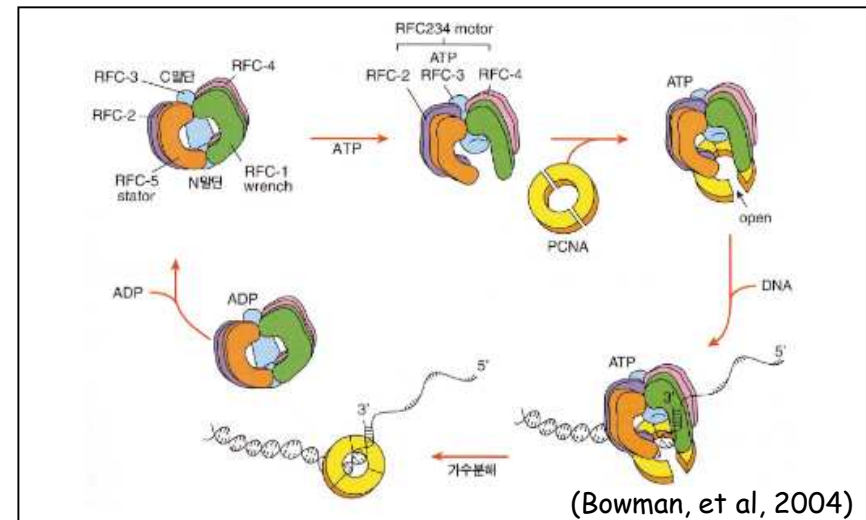


❖ RFC is?

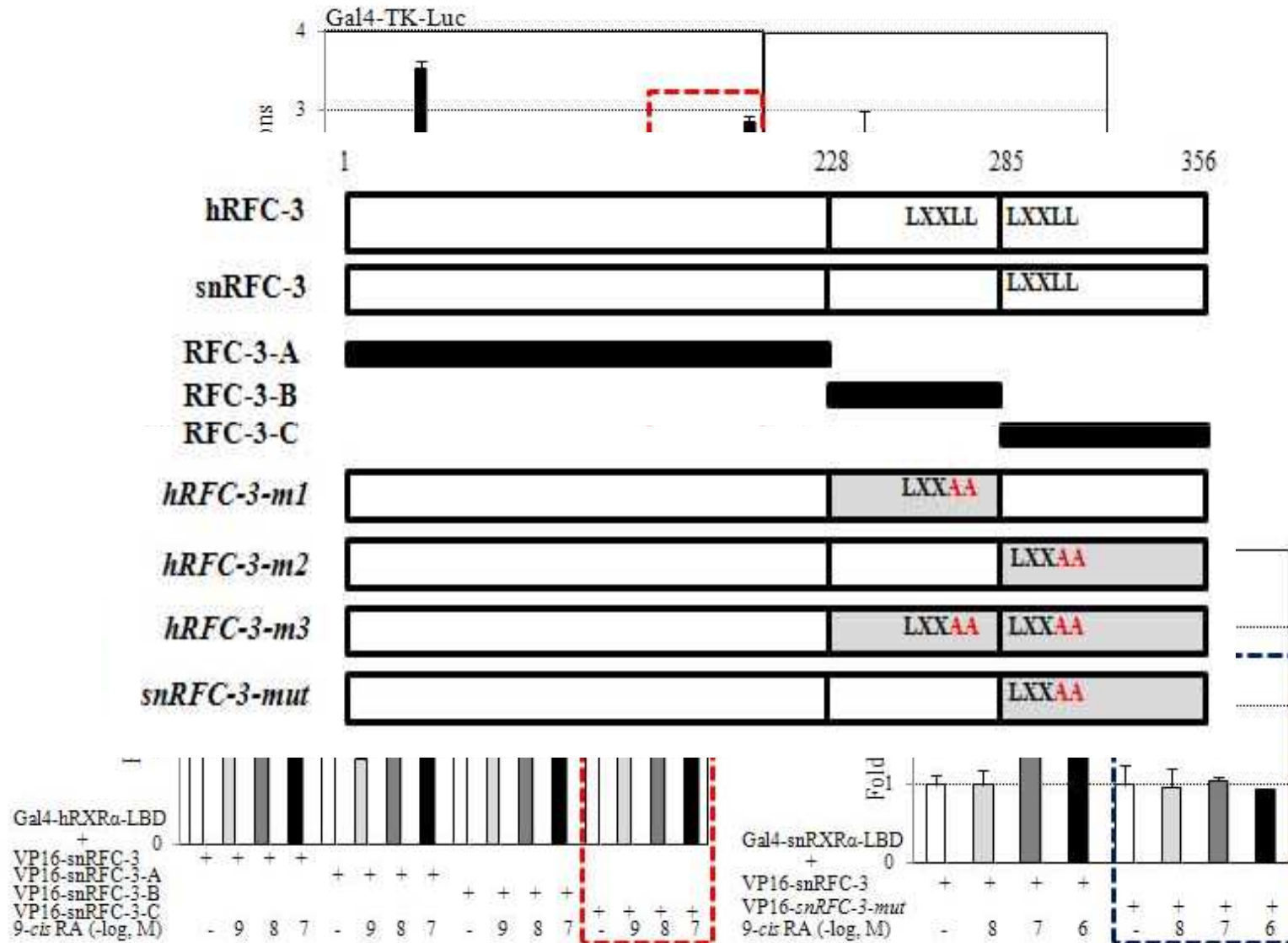
- Multiprotein complex consisting of one large and four small subunits.

- RFC has an associated ATPase activity

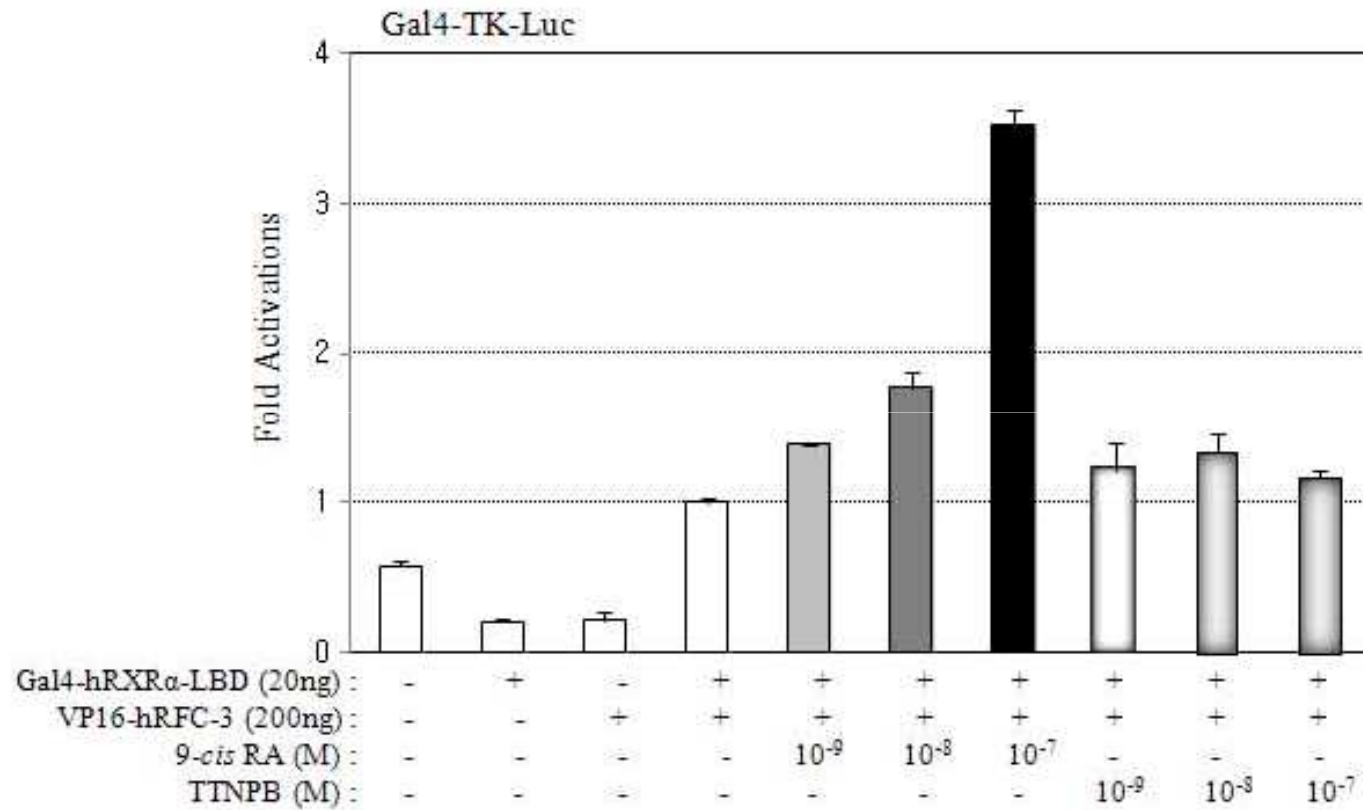
- It is a structure-specific DNA-binding protein and acts as a primer recognition factor for DNA polymerases δ and ϵ



Domain- and Motif-Specific Interaction

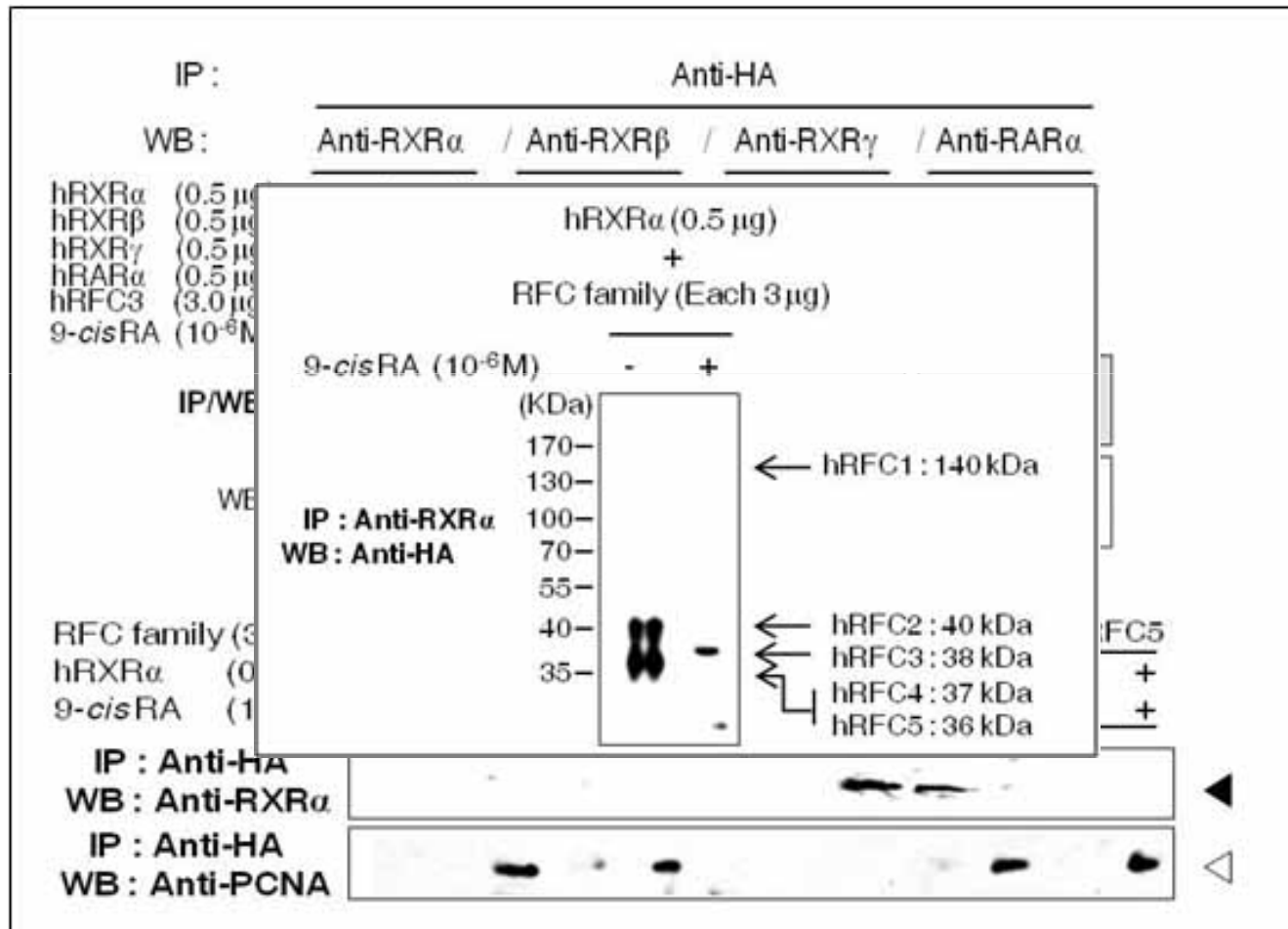


9-*cis* RA Specific Interaction

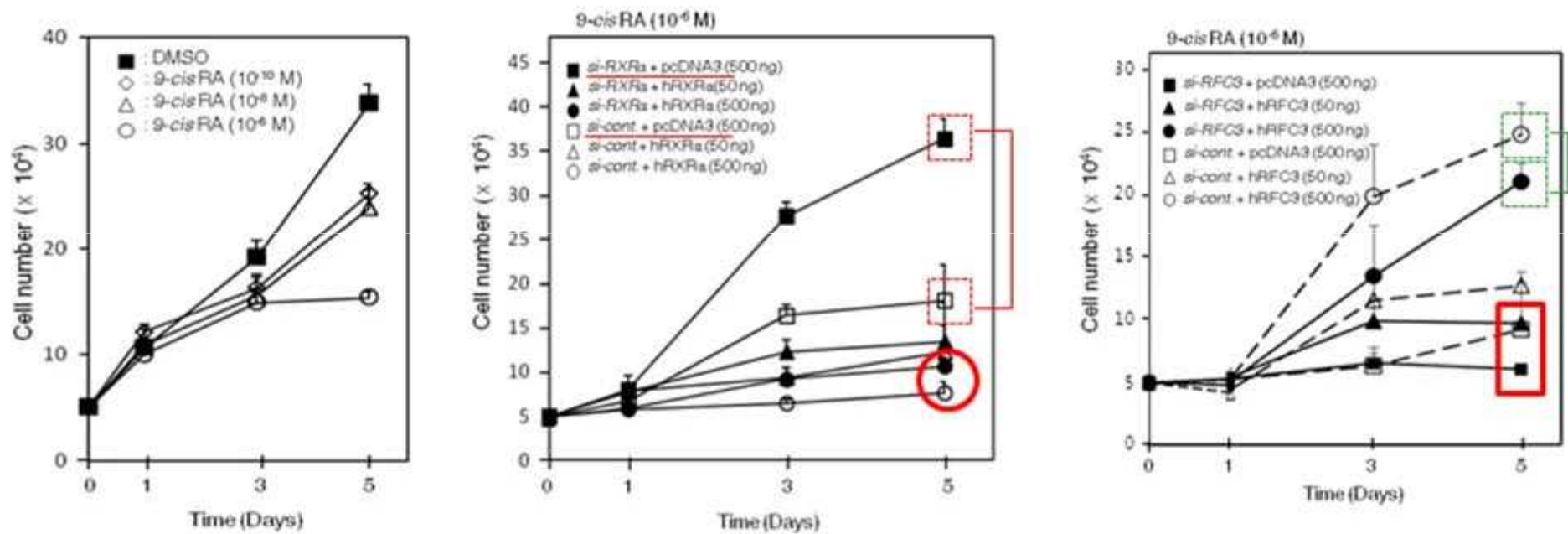


RXR α and RFC3-Specific Interaction

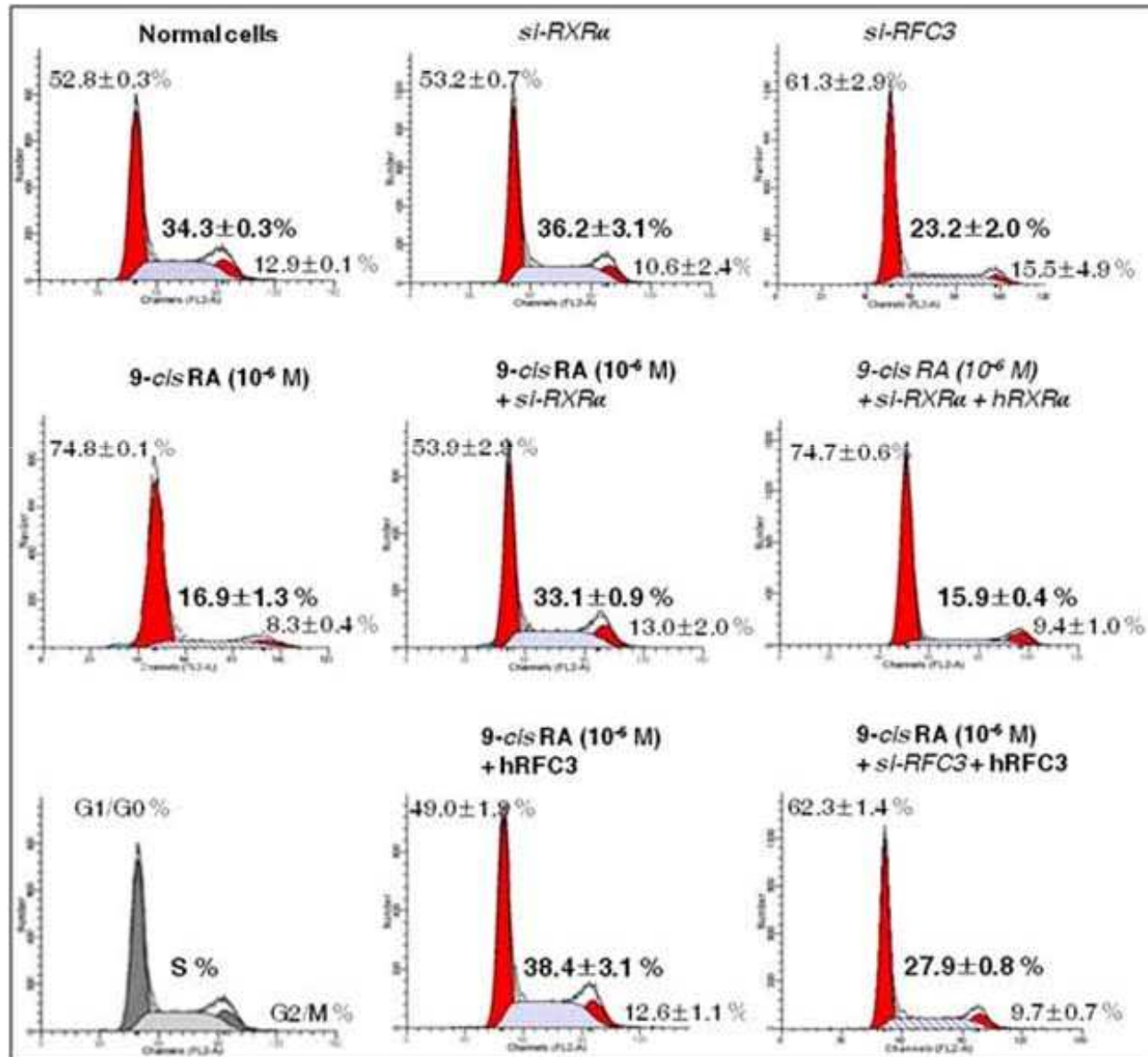
❖ *9-cis* RA-bound RXR results in reconfiguration of RFC-PCNA complex.



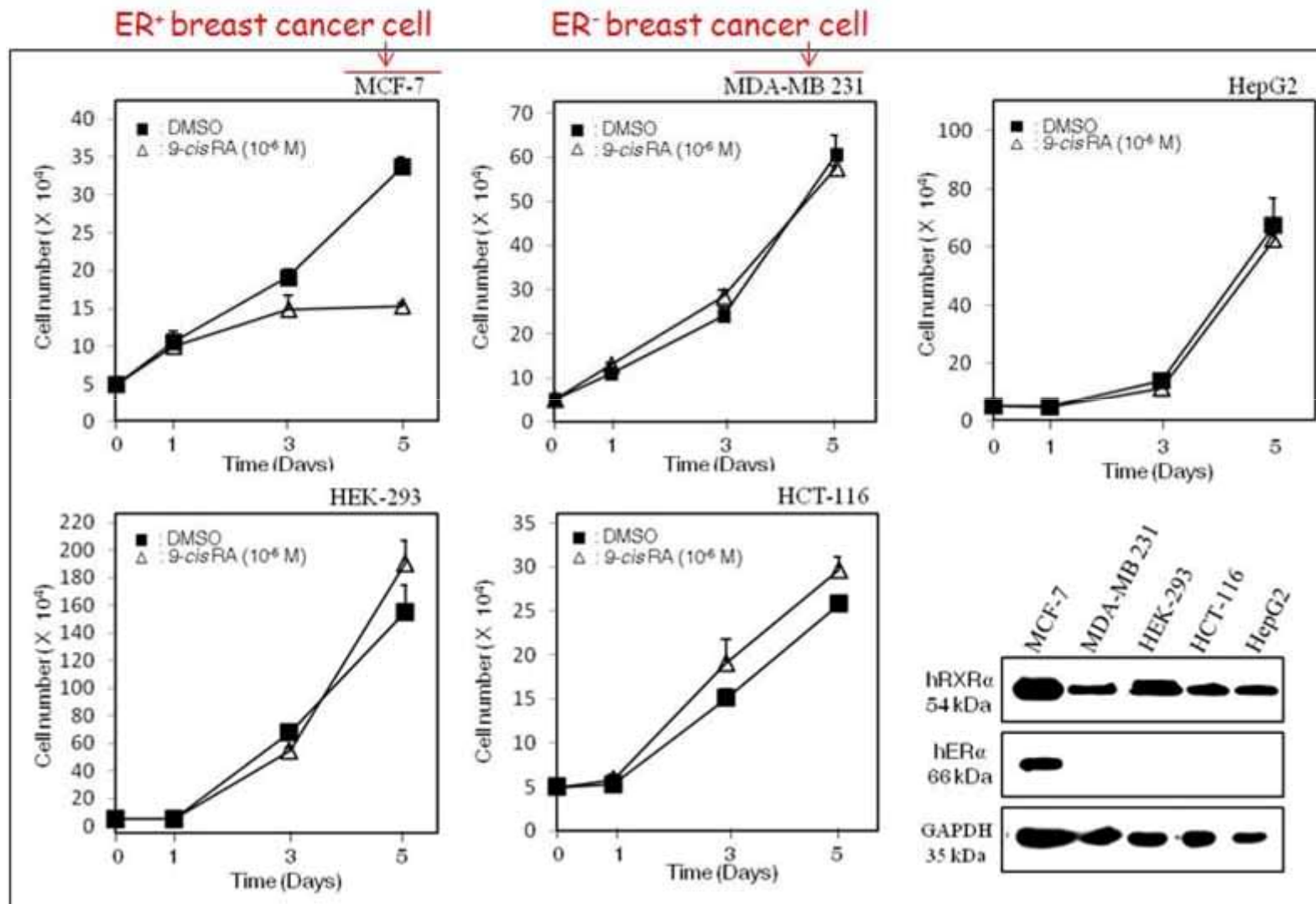
Knockdown of RXR α Recovers Proliferation of MCF-7 Cells Treated with 9-*cis* RA



Knockdown of RXR α Restores Cell Cycle Progression

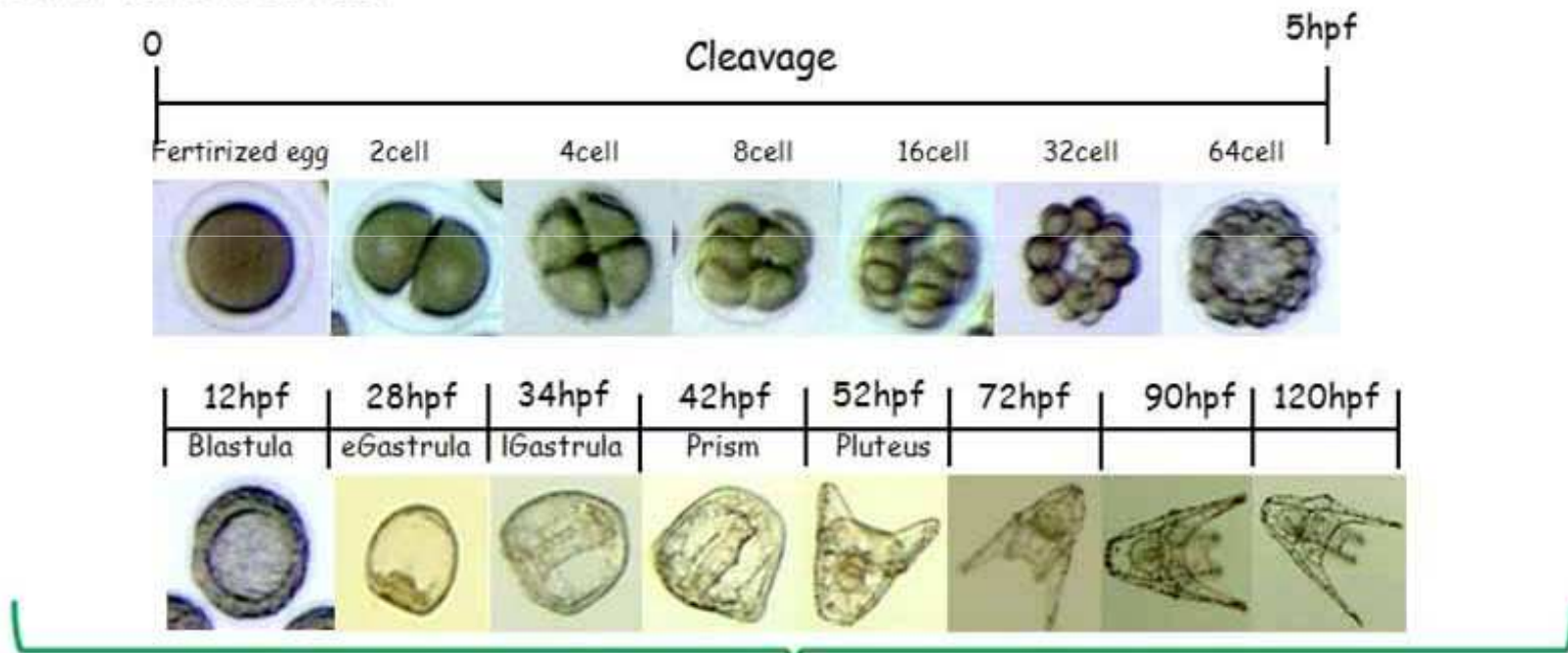


Growth profiles of several human cell lines responding to 9-*cis* RA





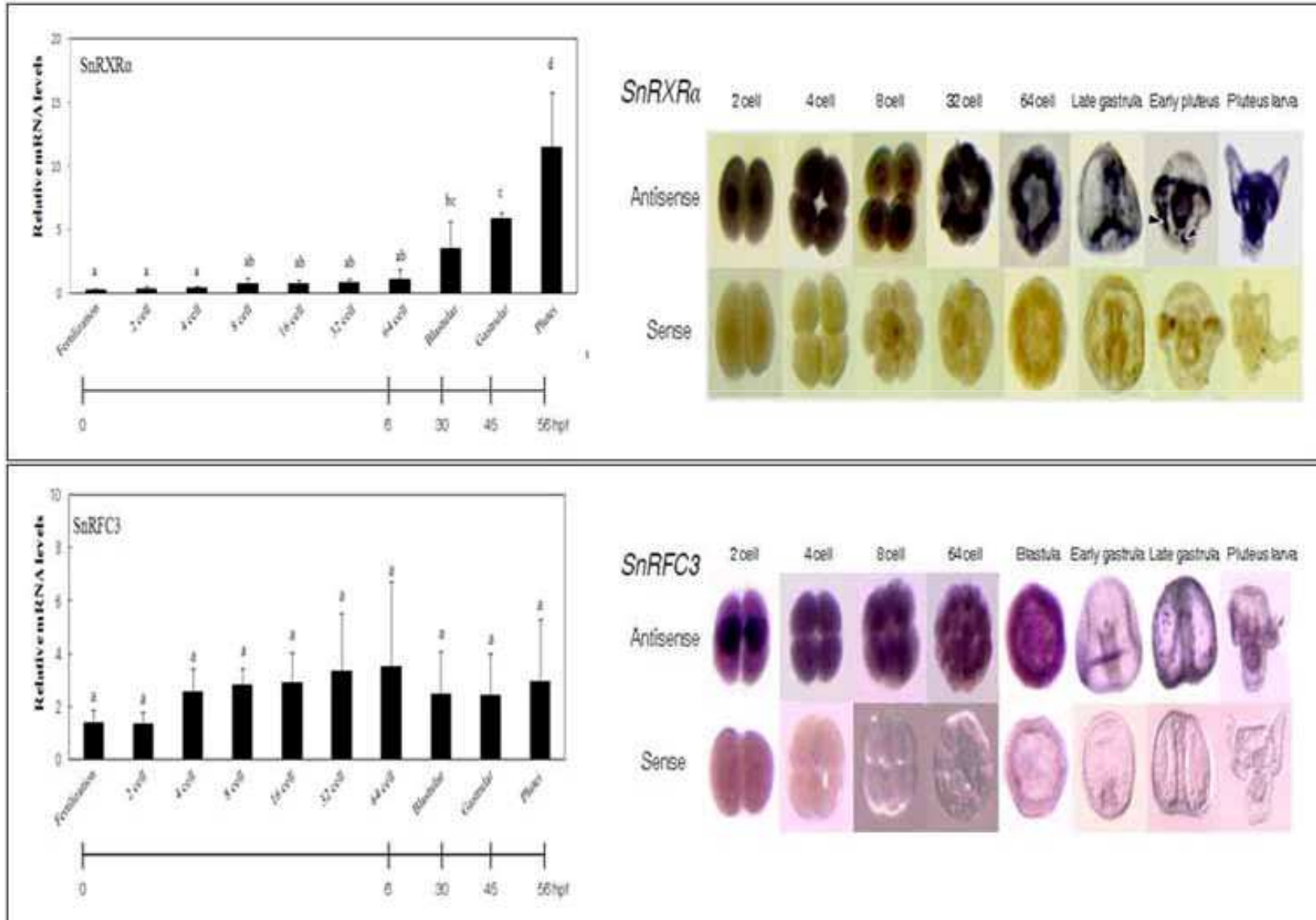
S. nudus for functional test



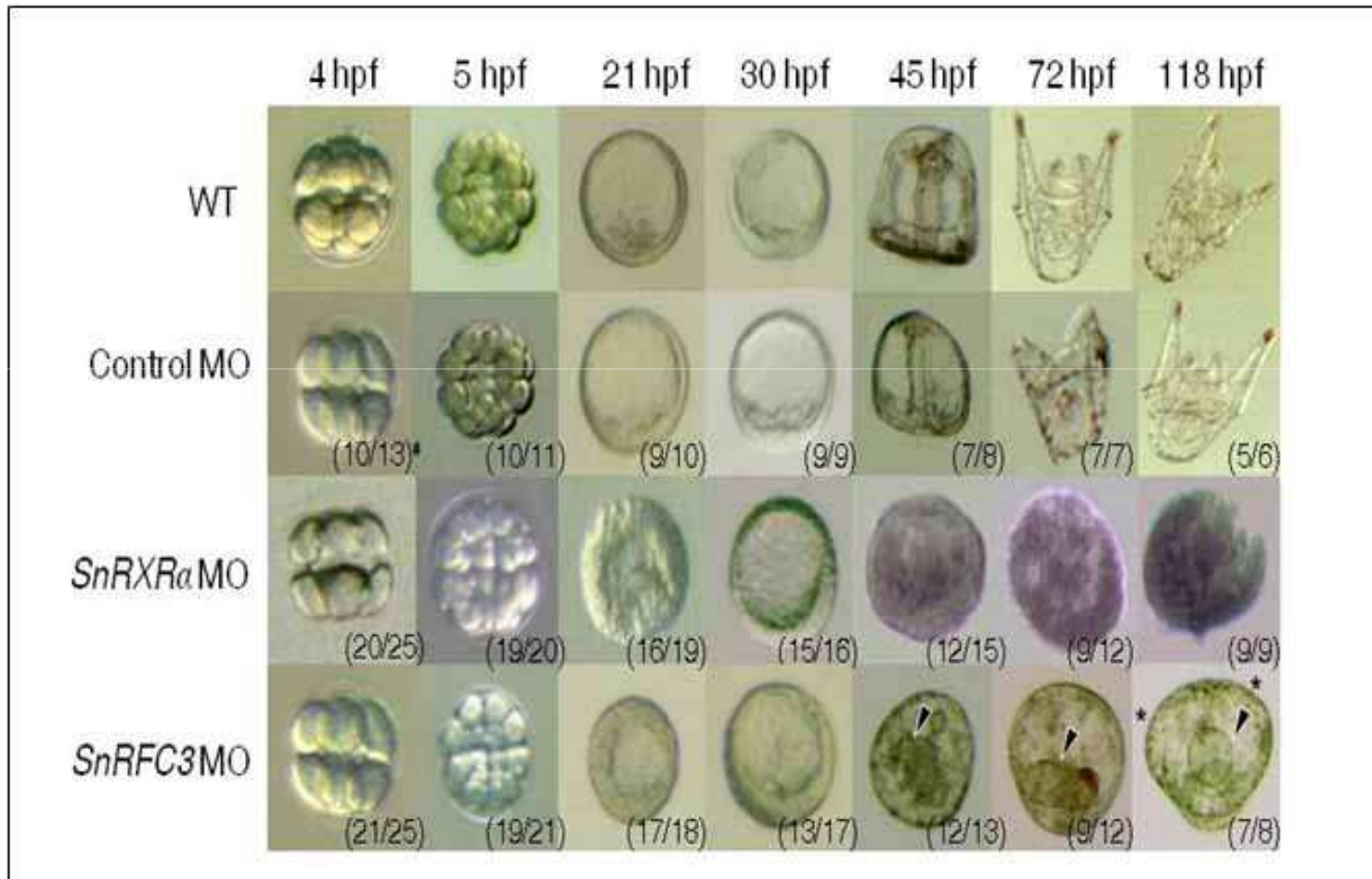
Quantitative real-time PCR analysis

Whole-mount in situ hybridization

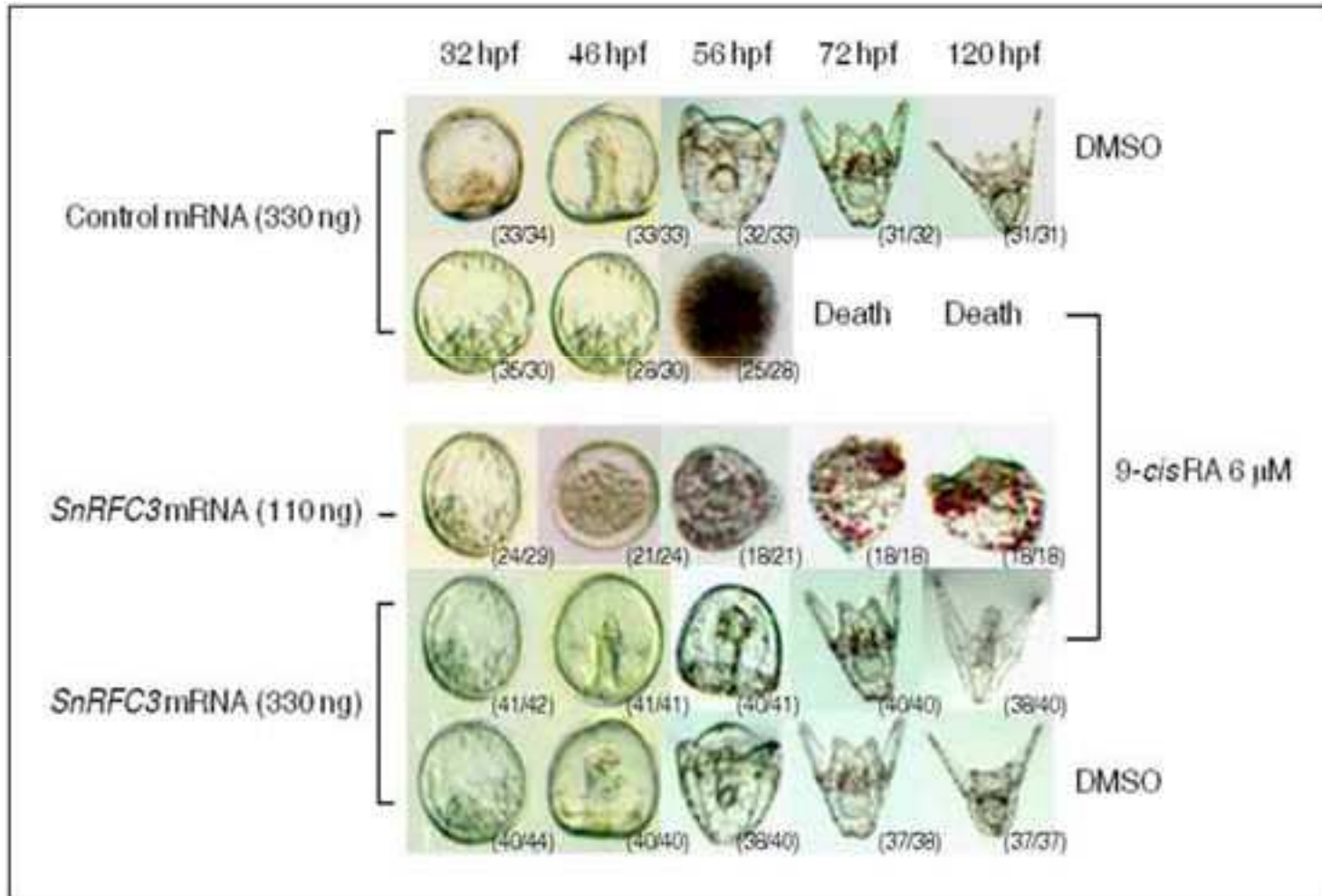
RXR α and RFC3 expression in *S. nudus*



Morphological changes of sea urchin embryos injected with RXRa morpholino in the presence of 9-*cis* RA (6 mM)

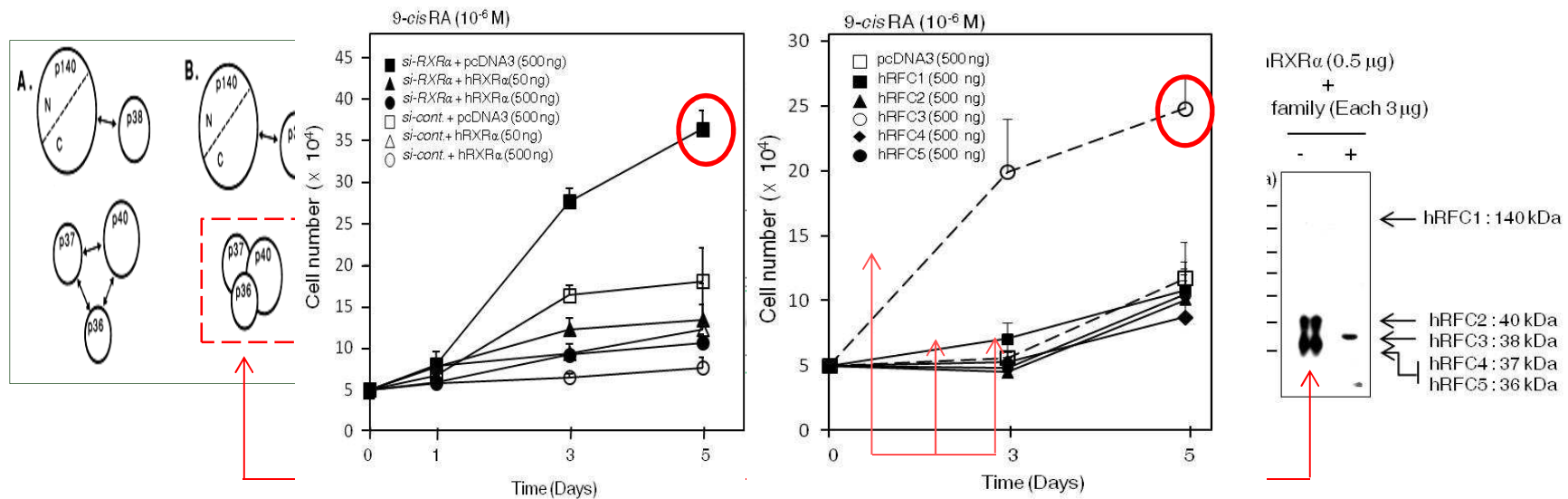
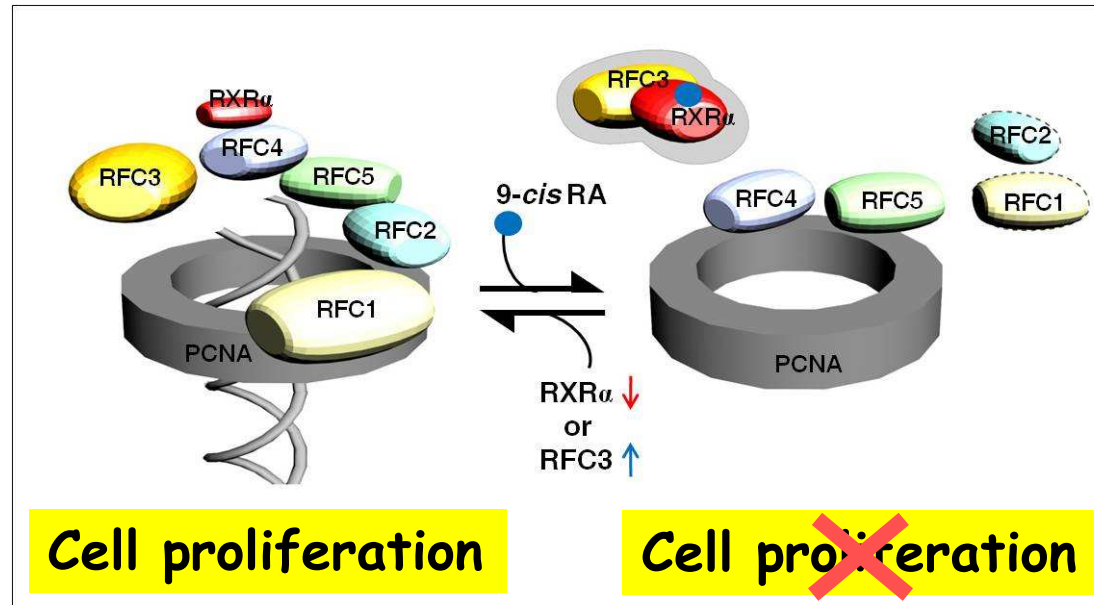


Morphological changes of sea urchin embryos injected with RFC3 morpholino



❖ Summary

Model for how 9-*cis* RA-activated RXR α may affect steady-state RFC-PCNA complex to inhibit cell proliferation



Molecular Endocrinology

9-Cis retinoic acid induces growth inhibition in retinoid-sensitive breast cancer and sea urchin embryonic cells via retinoid X receptor α and replication factor C3

--Manuscript Draft--

Manuscript Number:	ME-12-1104R2
Full Title:	9-Cis retinoic acid induces growth inhibition in retinoid-sensitive breast cancer and sea urchin embryonic cells via retinoid X receptor α and replication factor C3
Short Title:	Role of RXR α and RFC3 in retinoid-sensitive cells
Article Type:	Research Paper
Corresponding Author:	Young Chang Sohn, Ph.D. Gangneung-Wonju National University Gangneung, KOREA, REPUBLIC OF
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Gangneung-Wonju National University
Corresponding Author's Secondary Institution:	
First Author:	Sejung Maeng, Dr.
First Author Secondary Information:	
Order of Authors:	Sejung Maeng, Dr. Gil Jung Kim, Dr. Eun Ju Choi, Dr. Hyun Ok Yang, Dr. Dong-Sup Lee, Dr. Young Chang Sohn, Ph.D.

Acknowledgments

Prof. Sohn Young Chang



Prof. Kim Gil Jung



Prof. Dong-Sup Lee



Dr, Hyun Ok Yang



Dr, Eun Ju Choi



Prof. Jong-Man Yoon

한국발생생물학회

KOREAN SOCIETY OF DEVELOPMENTAL BIOLOGY

Thank you!!