SANE/XMan1 Regulates BMP Signaling through Interaction with Smad1/5

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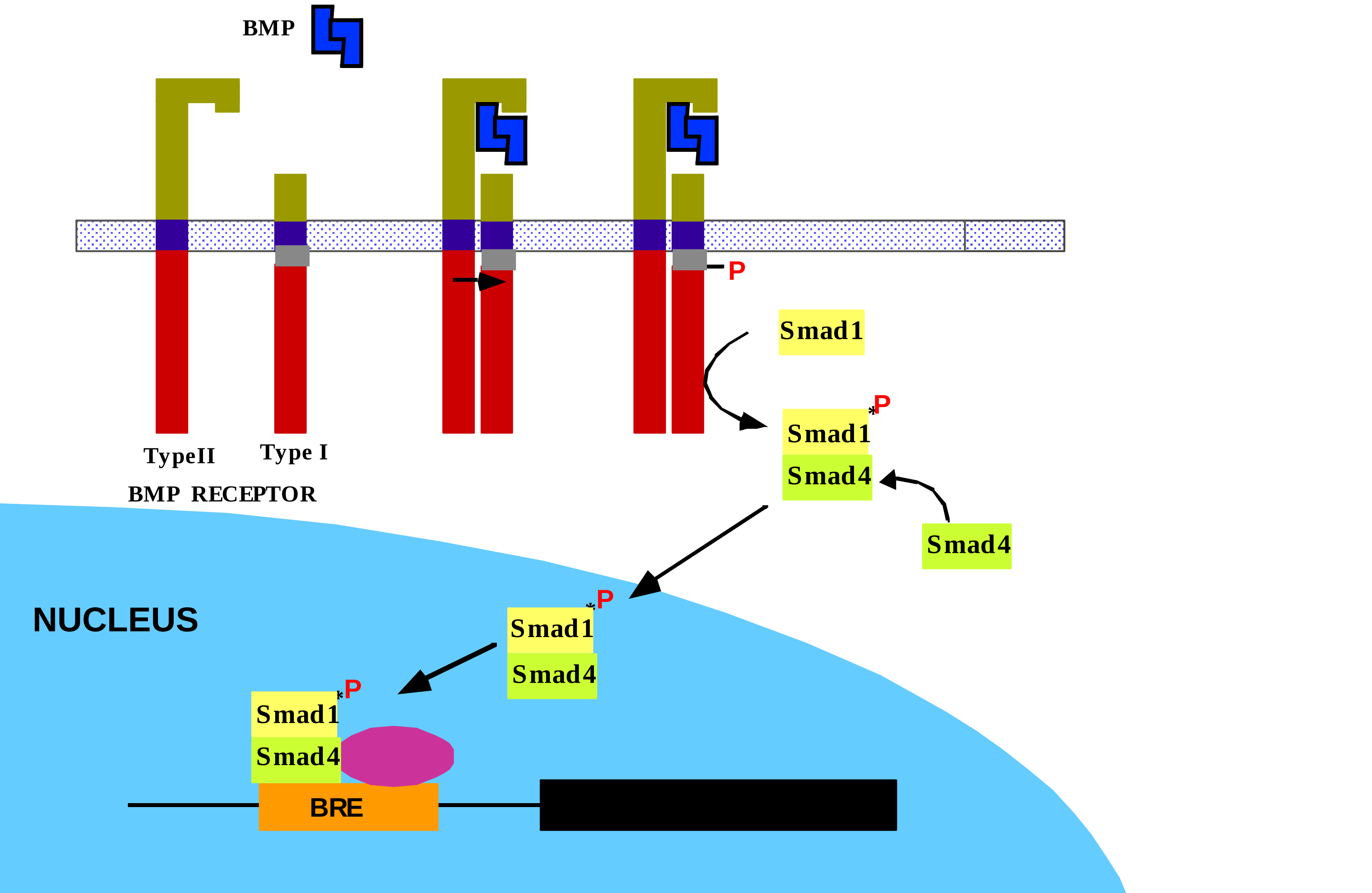
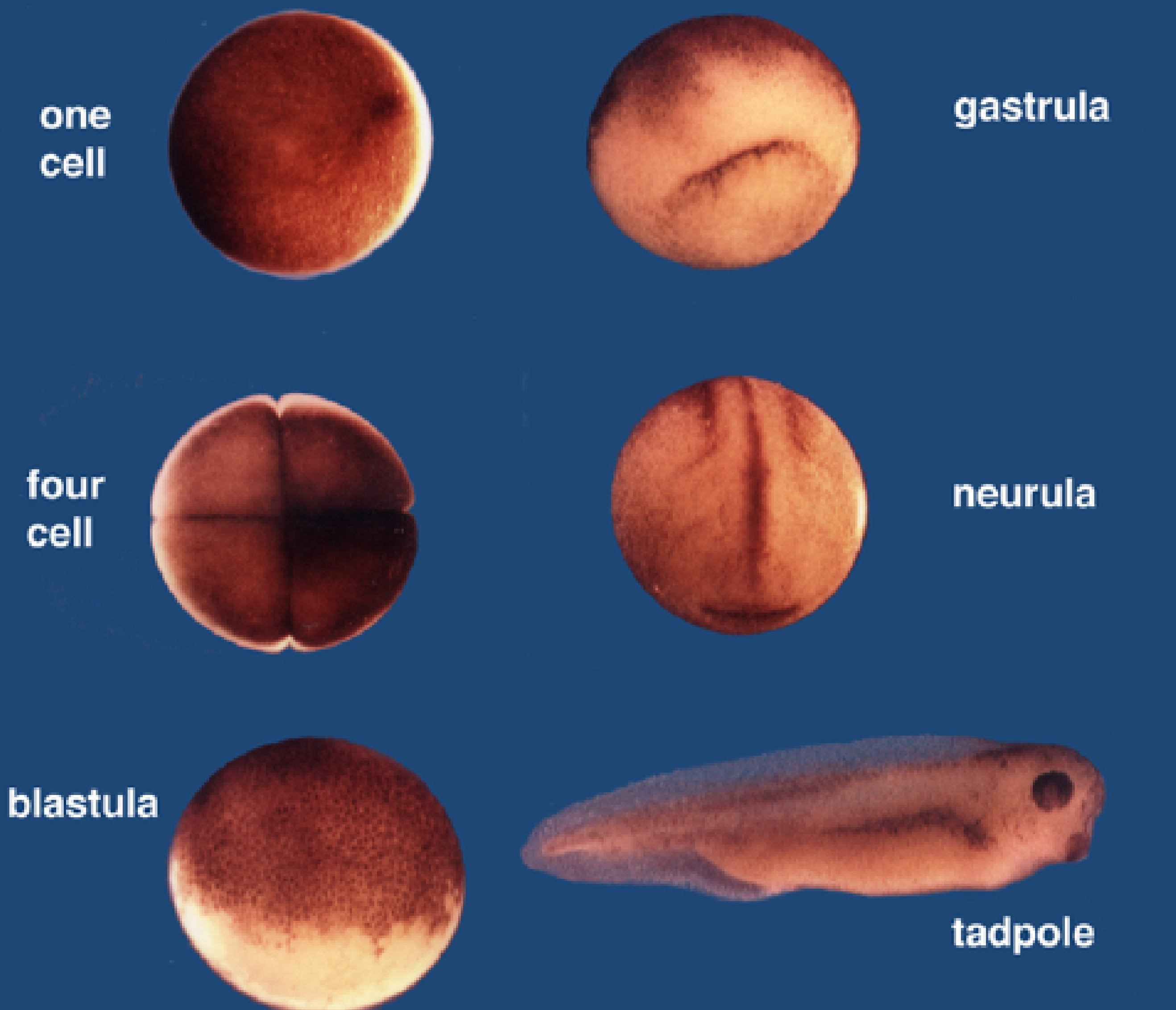
Functions of BMPs

- Bone formation
- Dorsal-ventral patterning
- Inhibition of neural induction
- Somite, neural patterning
- Many other developmental processes

BMPs Signal through Receptor Serine/ Threonine Kinases

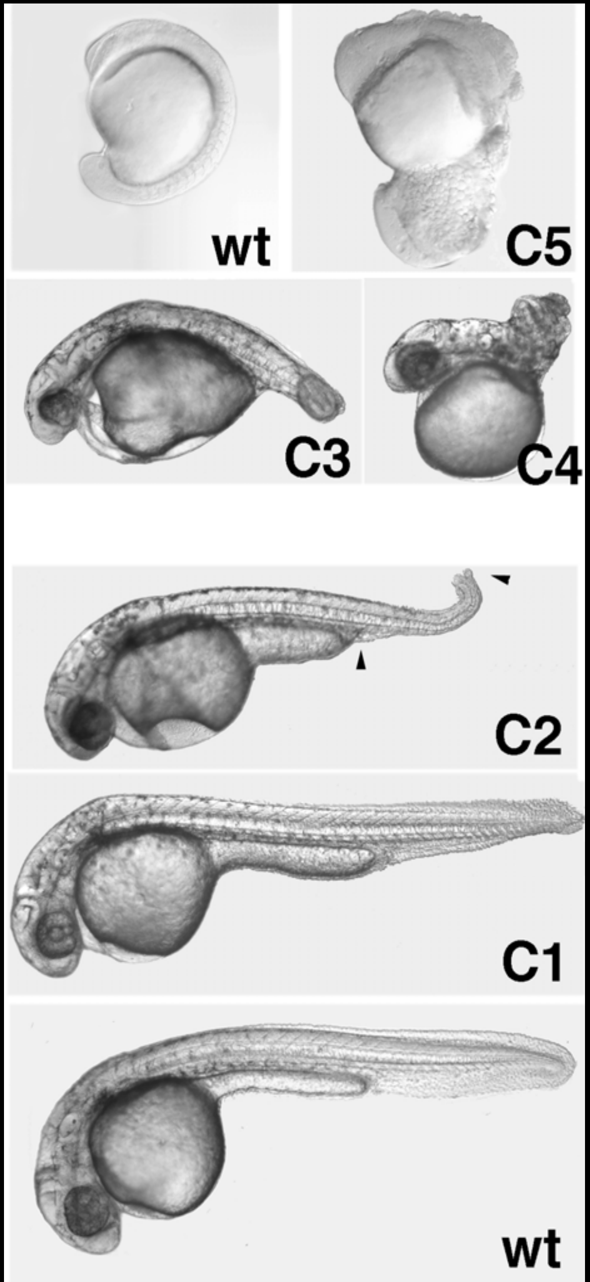


EARLY DEVELOPMENT OF XENOPUS LAEVIS



Loss of ventral signals dorsalizes *Xenopus* embryos

Zebrafish Dorsalized Mutants



swirl / BMP2b
somitabun / Smad5
snailhouse / BMP7 (null)
MZ-*lost-a-fin / Alk8*

piggytail / Smad5

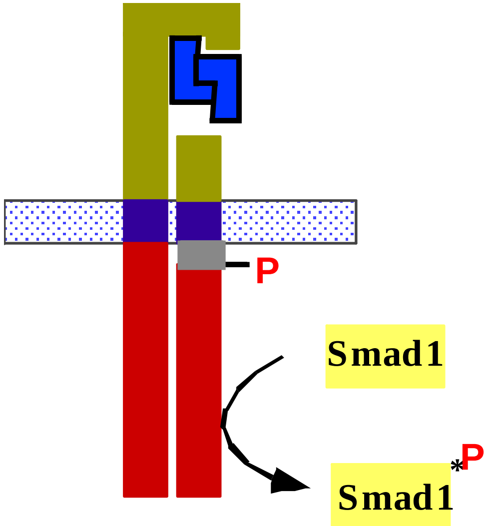
snailhouse / BMP7
(hypomorph)

Z-lost-a-fin / Alk8

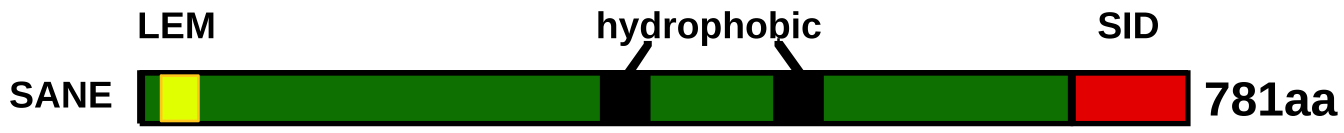
mini fin / Tolloid

wild type

Identification of proteins that interact with Smad1



SANE (Smad1 Antagonistic Effector)



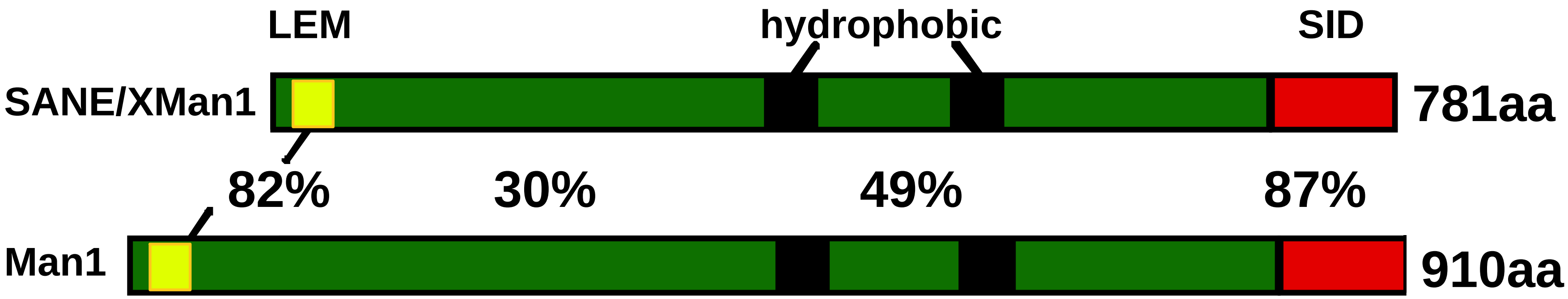
- **Identified in Yeast two-hybrid screen with XSmad1 as bait.**

(Raju et al JBC 2002)

SANE-MAN1 Sequence Alignment

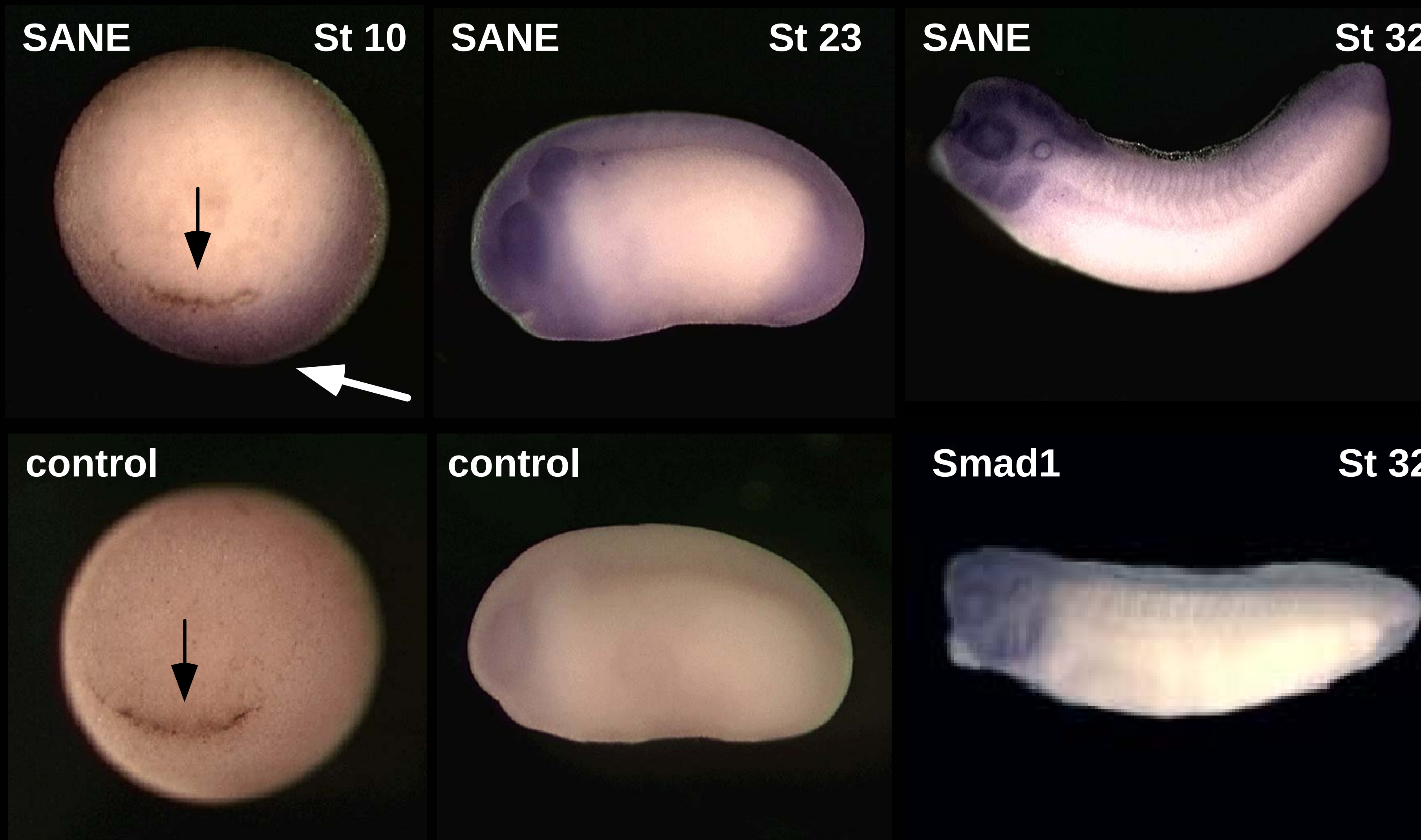
```
XSANE  MAAA------QLTDEELFSELKRSGIMPGPVTDSTRPVYL
hMAN1  MAAAAASAPQQLSDEELFSQLRRYGLSPGPVTESTRPVYL

XSANE  KKLKKLREEQRLRHG------KVRVLTNN-TAAAP-----
hMAN1  KKLKKLREEEQQQHRSGGRGNKTRNSNNNNTAAATVAAAG

XSANE  -----SESEGILKSGGDVTHKRYS------RQSPPR----
hMAN1  PAAAAAAGMGVRPVSGDLSYLRTPGGLCRISASGPESLLG

XSANE  -------EP-AKQSPILGFSSDESDADAG---LGAHGSRR
hMAN1  GPGGASAAPAAGSKVLLGFSSDESDVEASPRDQAGGGGRK

XSANE  VRANTPQS--ERKPGQLRATADANAD---RRKVPGWWEMR
hMAN1  DRASLQYRGLKAPPAPLAASEVTNSNSAERRKPHSWWGAR

XSANE  RPRG-------------DSEEEEDDEEEEEEEEGTEEASK
hMAN1  RPAGPELQTPPGKDGAVEDEEGEGEDGEERDPETEEPLWA

XSANE  CRTMNGS--RHSYCR-DYSDTDEEE---------LGKDEQ
hMAN1  SRTVNGSRLVPYSCRENYSDSEEEDDDDVASSRQVLKDDS

XSANE  QASRRSRRGSSK----------------------------
hMAN1  LSRHRPRRTHSKPLPPLTAKSAGGRLETSVQGGGGLAMND

XSANE  -QAKEQCMDR-----DDLPTWPGWSREVADVS--PRHRGA
hMAN1  RAAAAGSLDRSRNLEEAAAAEQGGGCDQVDSSPVPRYRVN

XSANE  ARKS-------HTAADS---DGAGNLKRKNNNHLESGYNK
hMAN1  AKKLTPLLPPPLTDMDSTLDSSTGSLLKTNNHIGGGAFSV

XSANE  DPPRVYFPSNSLPPRMPQSN----HTAAPNHTKYSHYHYP
hMAN1  DSPRIYS--NSLPPSAAVAASSSLRINHANHTGSNHTYLK

XSANE  DSKRQHHKVAEPEEALLQQFKRDEVSAAGRFSAHYLSMFL
hMAN1  NTYN-KPKLSEPEEELLQQFKREEVSPTGSFSAHYLSMFL

XSANE  LTAACLFFLLLGLTYLRMRGTSLPAN-----ENLTSTEDL
hMAN1  LTAACLFFLILGLTYLGMRGTGVSEDGELSIENPFGETFG

XSANE  NVDQS-KKNLMNTLQKLHEKLSKTAGDYICGNTDHRNISV
hMAN1  KIQESEKTLMMNTLYKLHDRLAQLAGDHECGSSSQRTLSV

XSANE  QEAKQYLRTLGQEYENEFNNTLAWILEHSATRDIGIKCVS
hMAN1  QEAAAYLKDLGPEYEGIFNTSLQWILEN--GKDVGIRCVG

XSANE  D-PGS-ILNITSVKYLESTLPKMSFMCRIKRAVITVAHRS
hMAN1  FGPEEELTNITDVQFLQSTRPLMSFWCRFRRAFVTVTHRL

XSANE  SILLVGVGVLFGVWRYIKYRWAKEEEETKQMYDMVVKIID
hMAN1  LLLCLGVVMVCVVLRYMKYRWTKEEEETRQMYDMVVKIID

XSANE  VLKSHNEACQENKGLEPYTPIPHVRDSLILPQDRKKMKKV
hMAN1  VLRSHNEACQENKDLQPYMPIPHVRDSLIQPHDRKKMKKV

XSANE  WDRAVEFLDANESRVRTETQKIGGADFQVWKWIQPSSTCD
hMAN1  WDRAVDFLAANESRVRTETRRIGGADFLVWRWIQPSASCD

XSANE  KISVMPSKVWQGQAFHLDRRNSPPNSLTPCLKIRNMFDPV
hMAN1  KILVIPSKVWQGQAFHLDRRNSPPNSLTPCLKIRNMFDPV

XSANE  MEIGDHWDLAIQEAILEKCSDNEGIVHIAVDKNSREGCVY
hMAN1  MEIGDQWHLAIQEAILEKCSDNDGIVHIAVDKNSREGCVY

XSANE  VKCLSPEFAGKAFKALHGSWFDGKLVTVKYLRLDRYHHRF
hMAN1  VKCLSPEYAGKAFKALHGSWFDGKLVTVKYLRLDRYHHRF

XSANE  PQALTCSTPLKPSNTHMNSLLHLNLRTGTSNSQSCS
hMAN1  PQALTSNTPLKPSNKHMNSMSHLRLRTGLTNSQGSS
```

SANE (Smad1 Antagonistic Effector)/XMan1

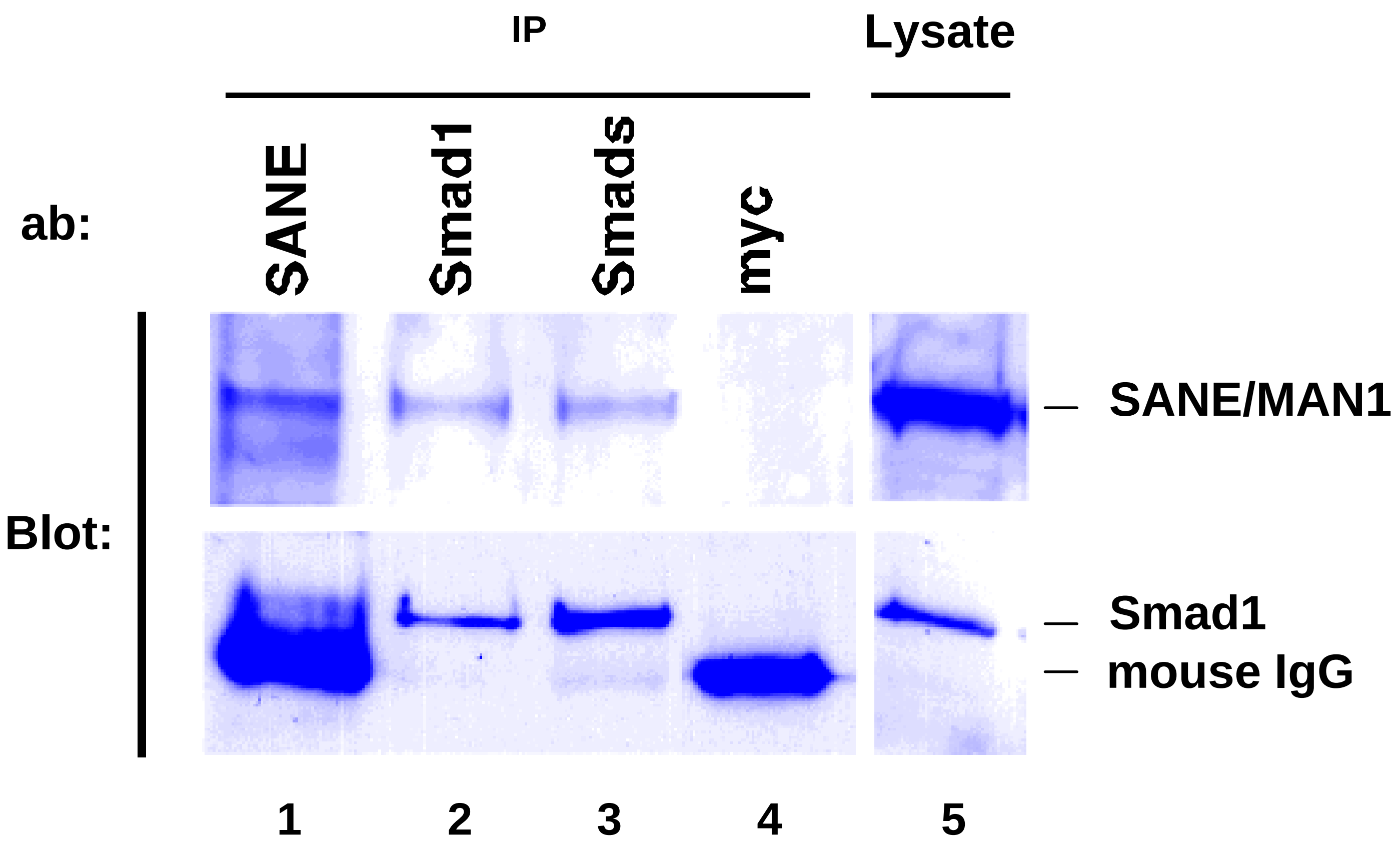


- **Identified in Yeast two-hybrid screen with XSmad1 as bait.**
- **SANE: Raju et al JBC 2002**
- **XMAN1: Osada et al Development 2003**
- **Interacts with Smad1/5 through C terminal SID**

Expression of SANE/XMan1 in Xenopus embryos

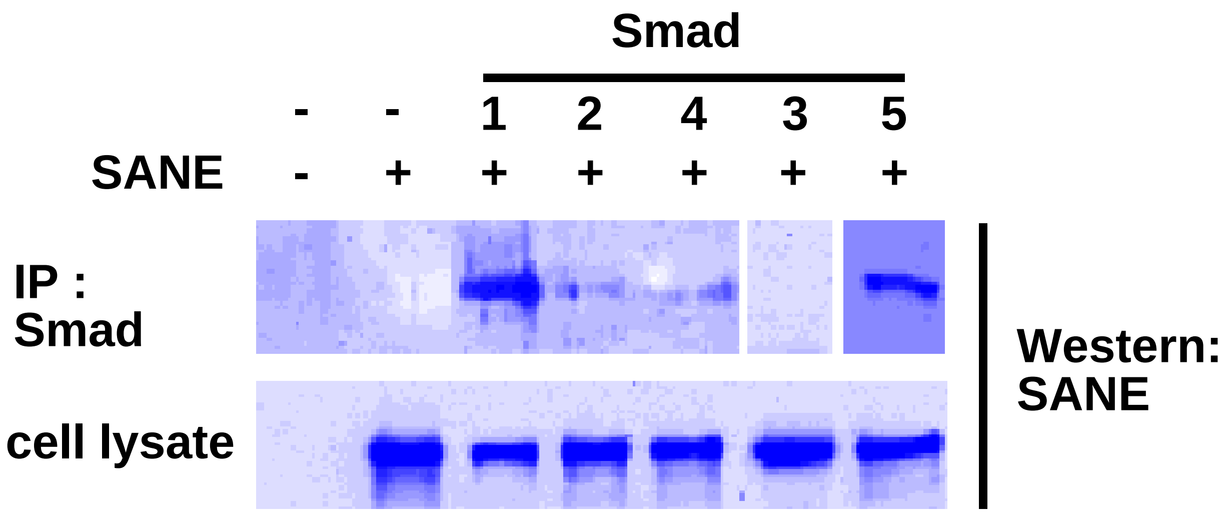


SANE/XMan1 interacts with Smad1



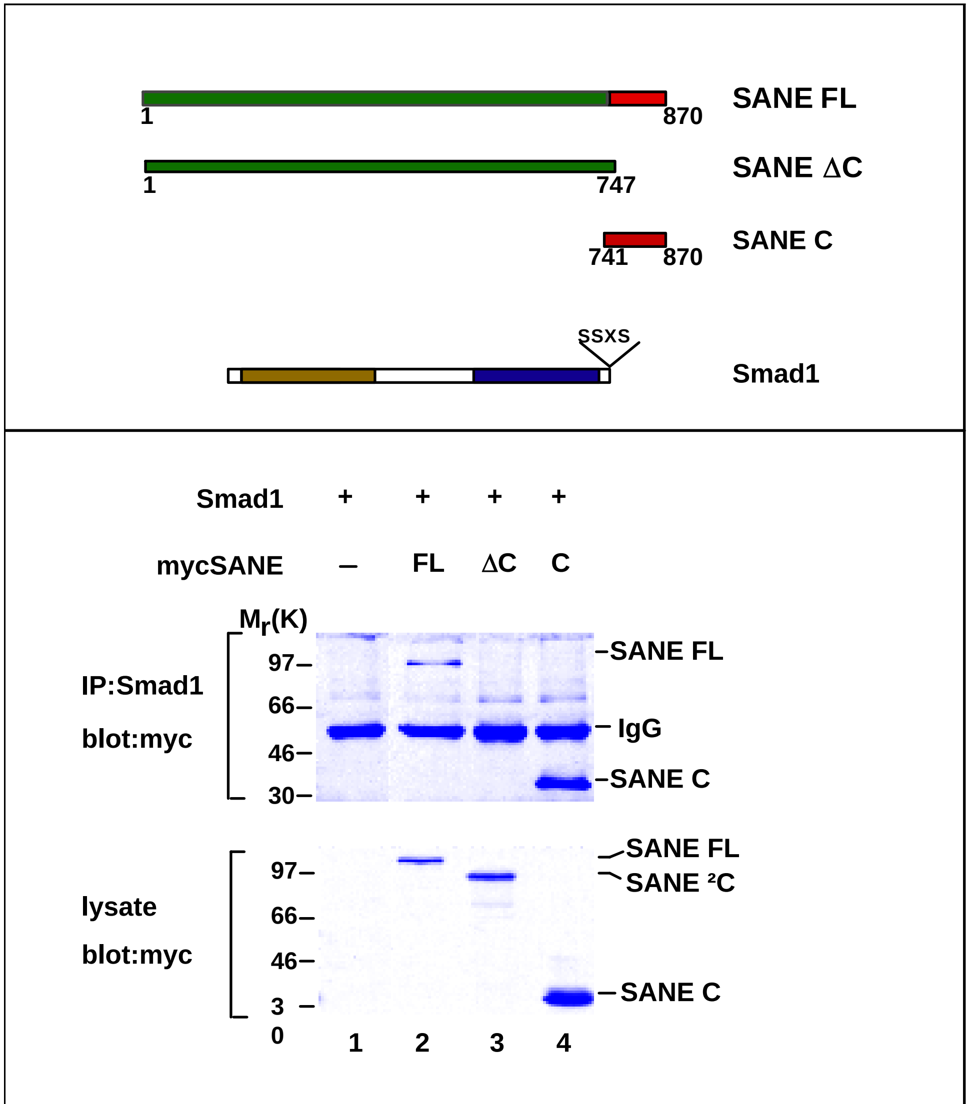
(endogenous proteins)

SANE/XMan1 interacts with Smads 1 & 5 and weakly with Smads 2 and 4



(in Xenopus…
but see Lin et al and Pan et al 2005 papers)

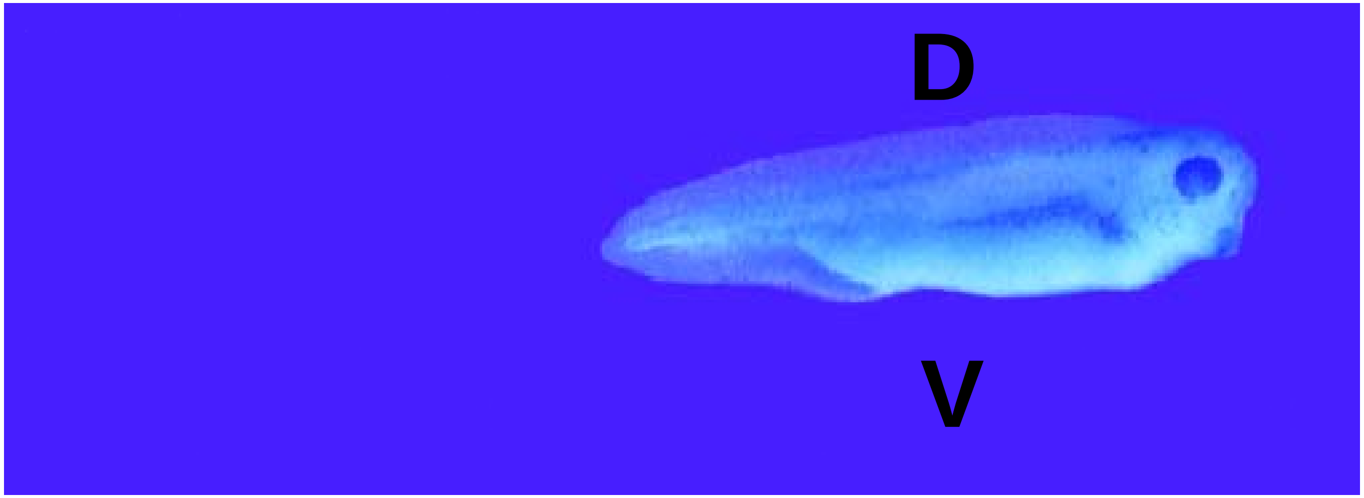
Smad1 interaction domain of SANE is in the C terminal region (SANE-C)



Summary:

- SANE/XMan1 binds BMP specific Smads (Smad1 and Smad5)

Dorsal-ventral patterning by BMPs is established in the early embryo



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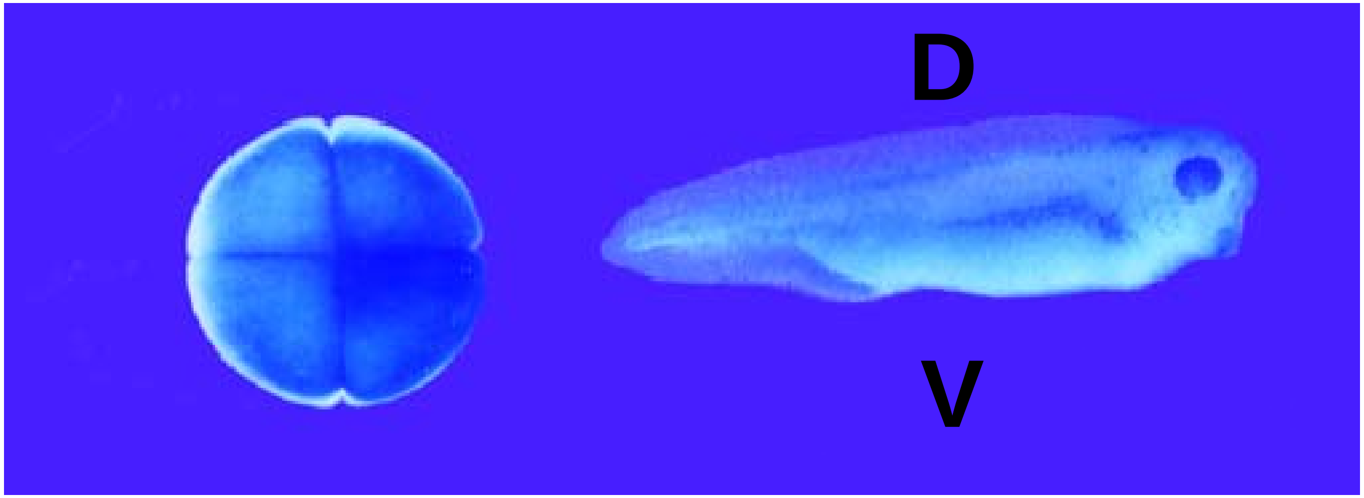
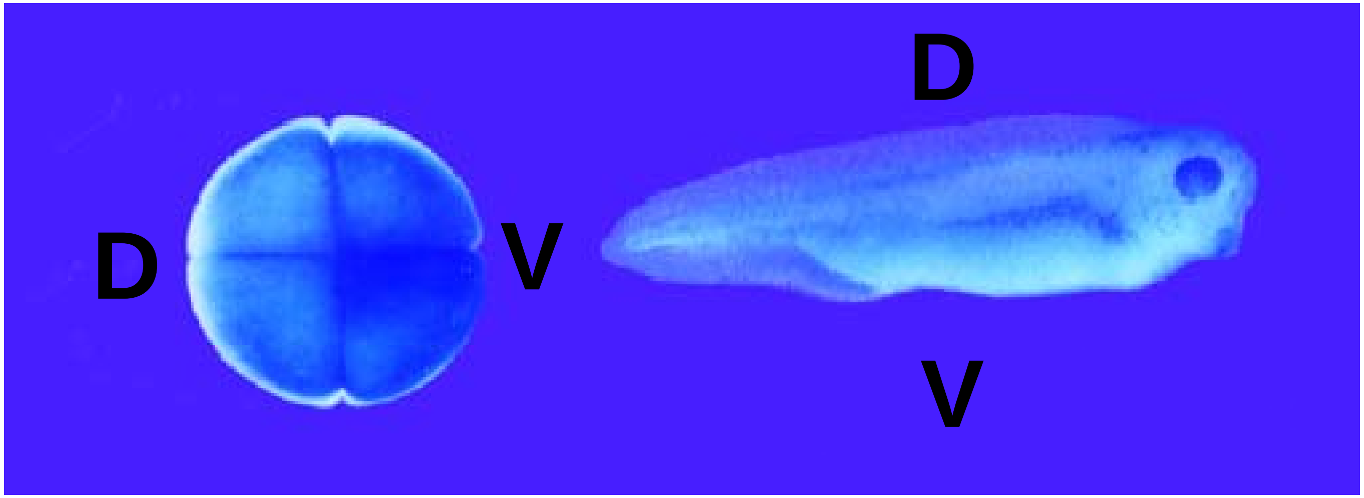
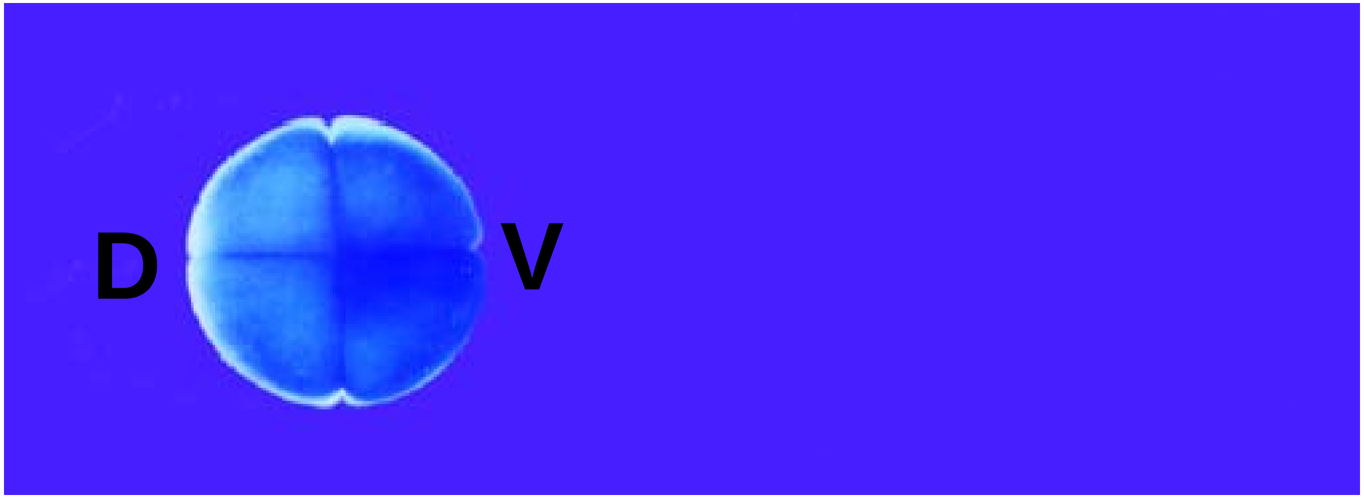


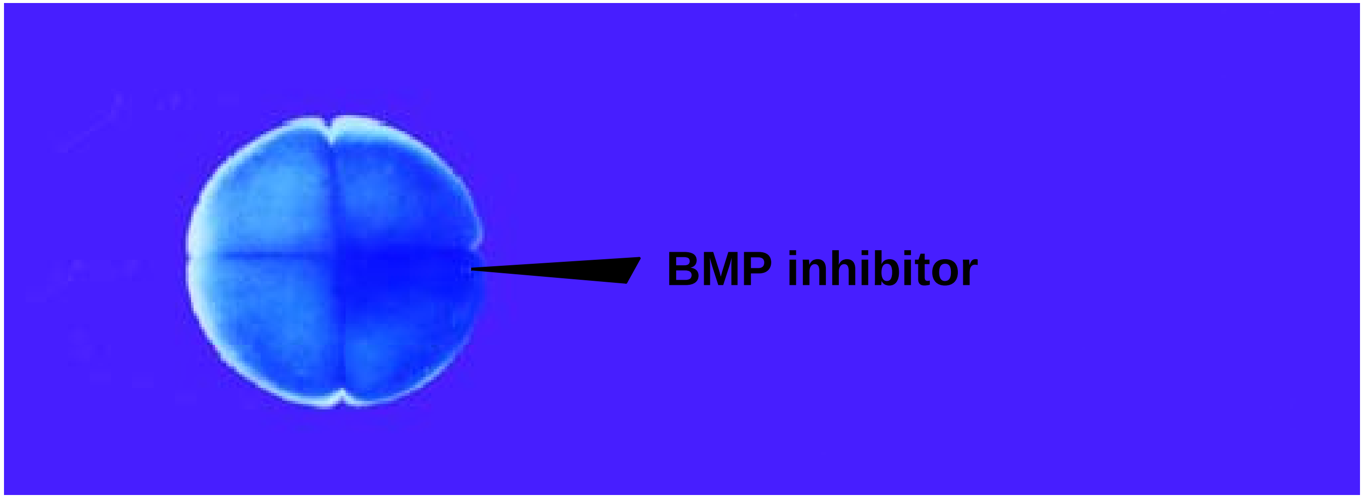
Dorsal-ventral patterning by BMPs is established in the early embryo



Dorsal-ventral patterning by BMPs is established in the early embryo

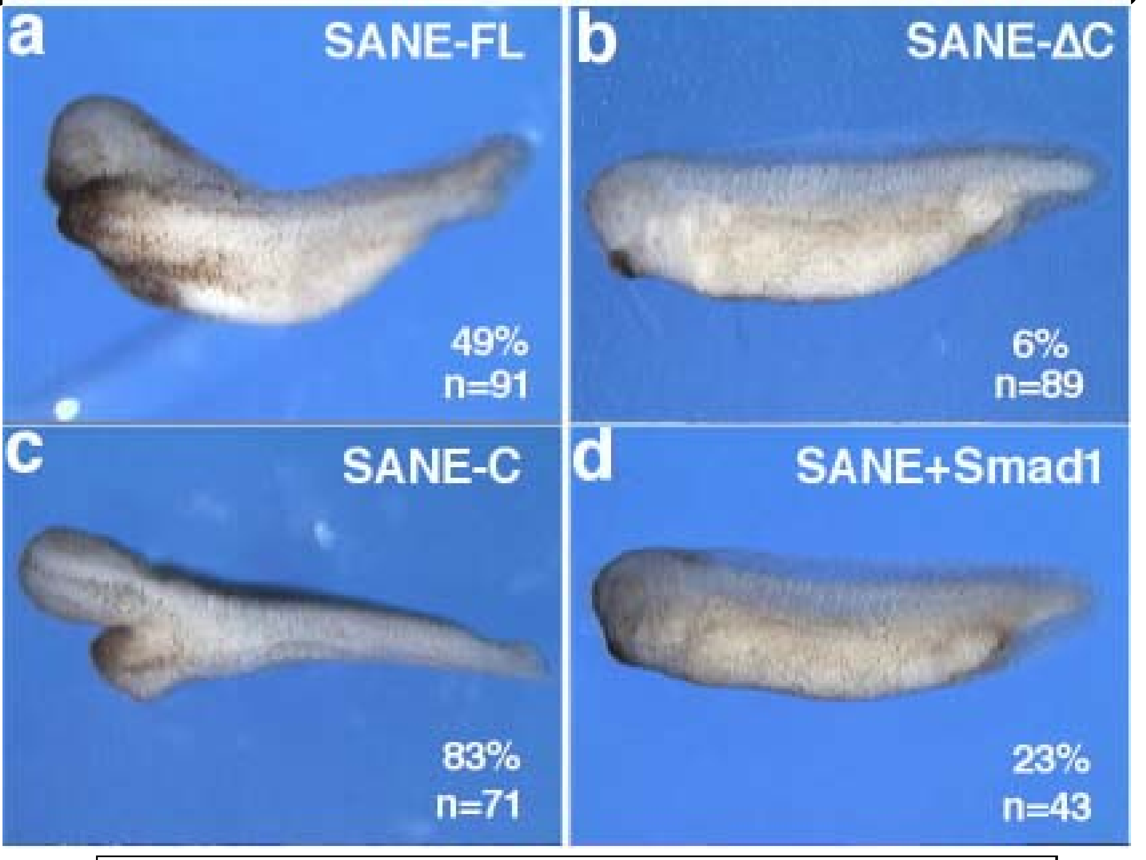


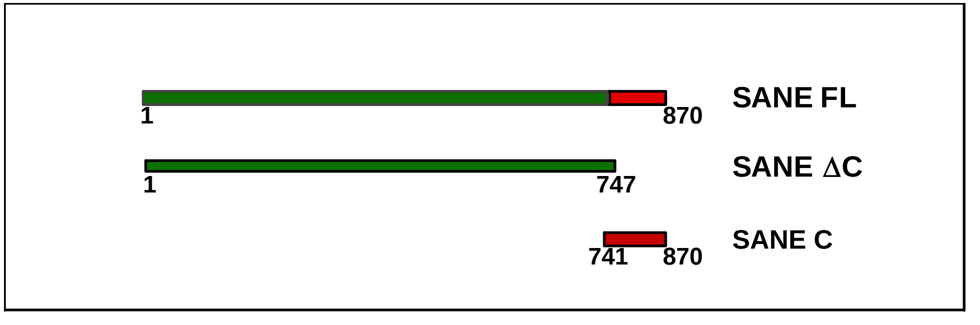
Dorsal-ventral patterning by BMPs is disrupted by BMP inhibitors



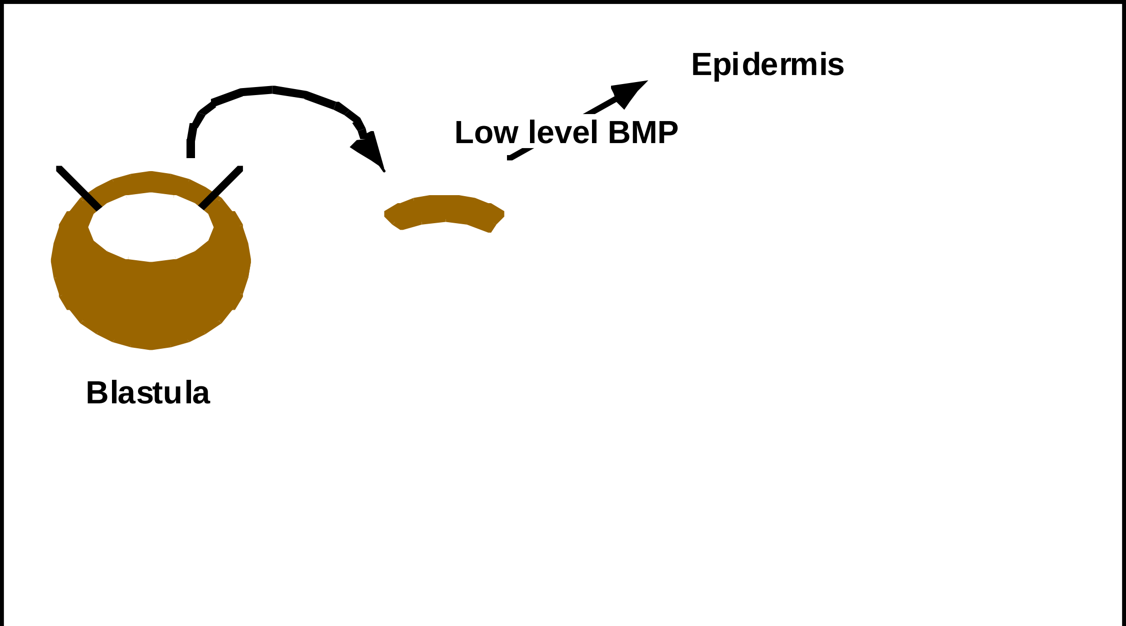
<u>BMP inhibitors</u>:
Chordin, noggin, follistatin
Dominant negative BMPR;
BMP loss of function mutations in zebrafish

SANE/XMan1 antagonizes BMP signaling in Xenopus embryos

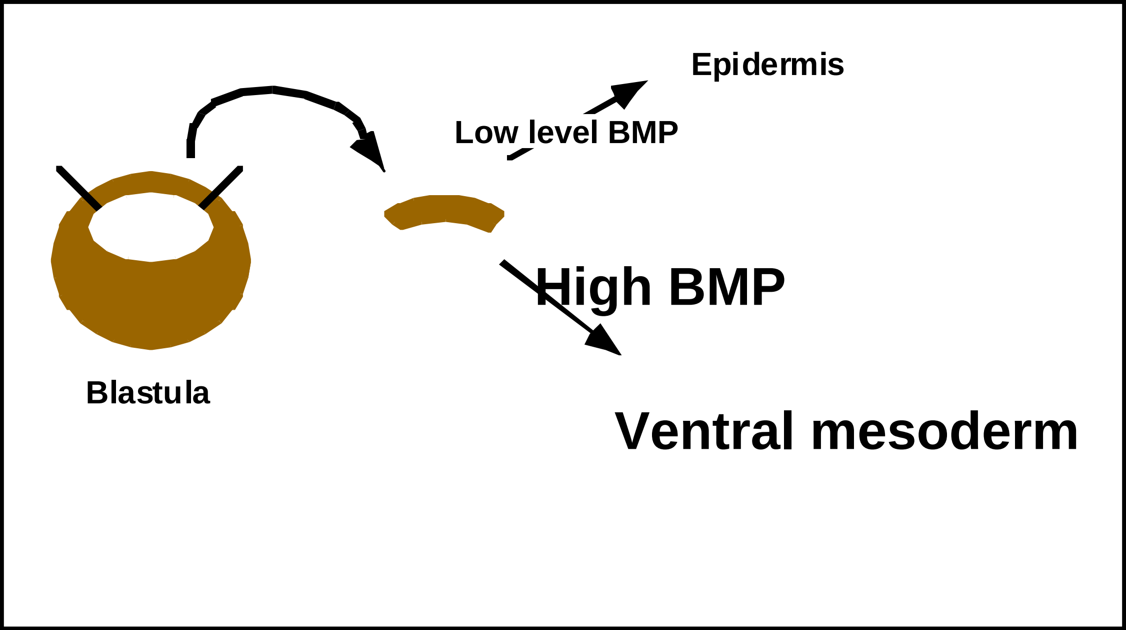




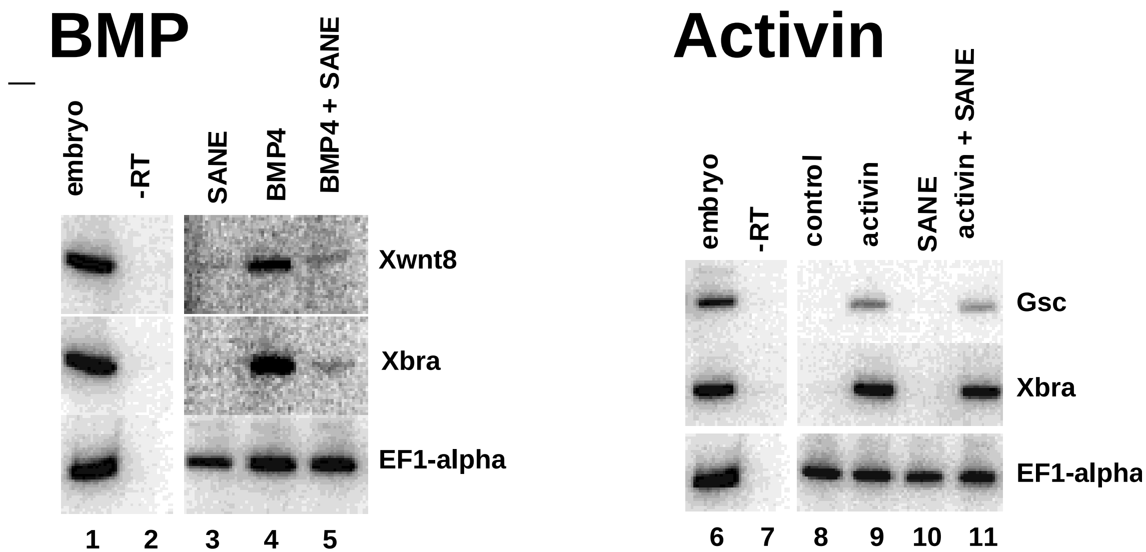
Mesoderm induction in ectodermal explants (animal cap assay)



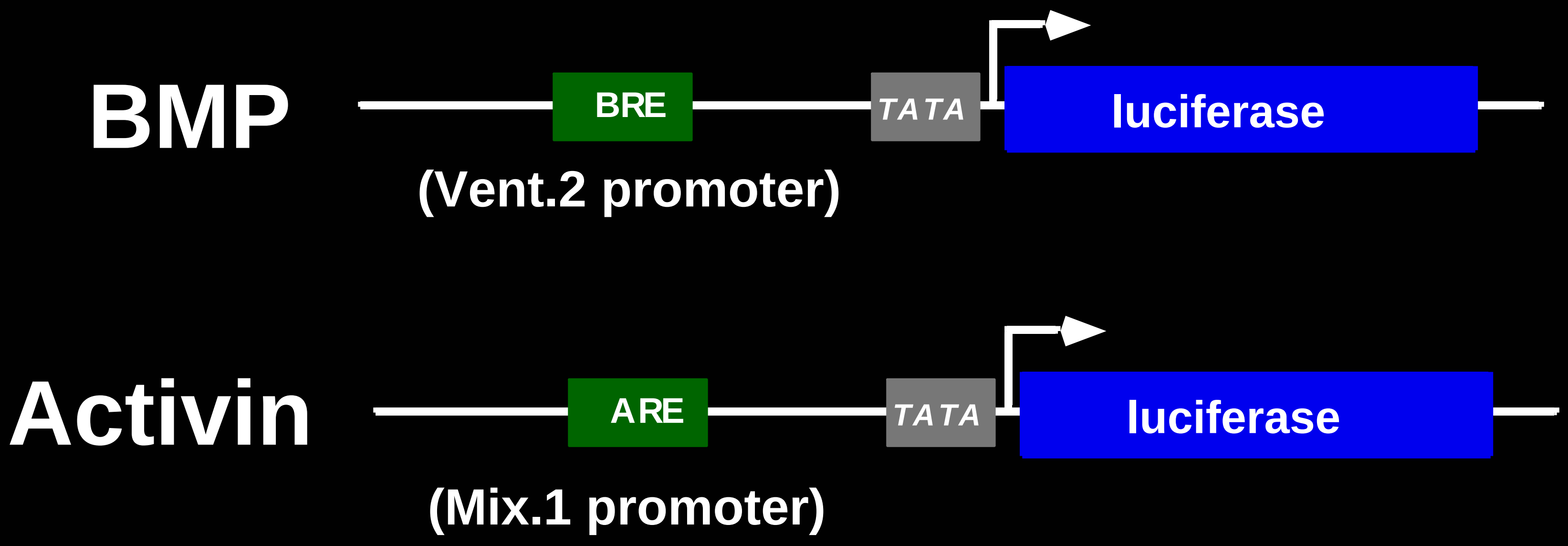
Mesoderm induction in ectodermal explants (animal cap assay)



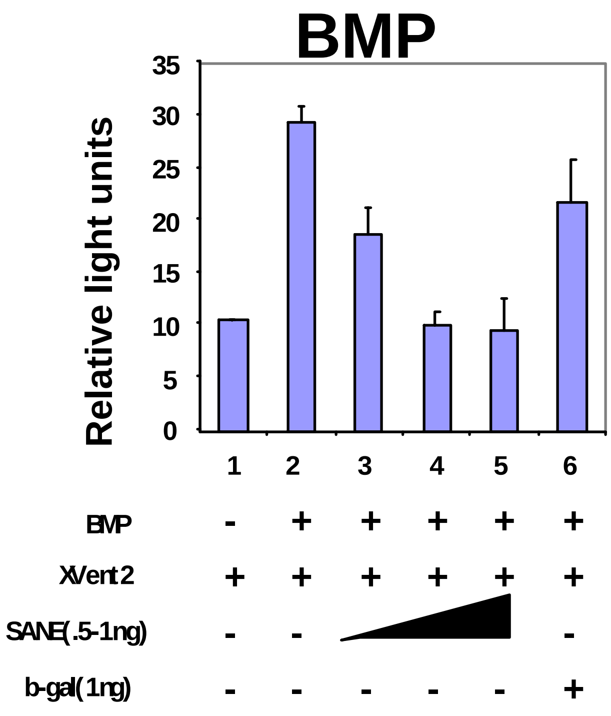
SANE/Xman1 inhibits ventral mesodermal induction by BMP

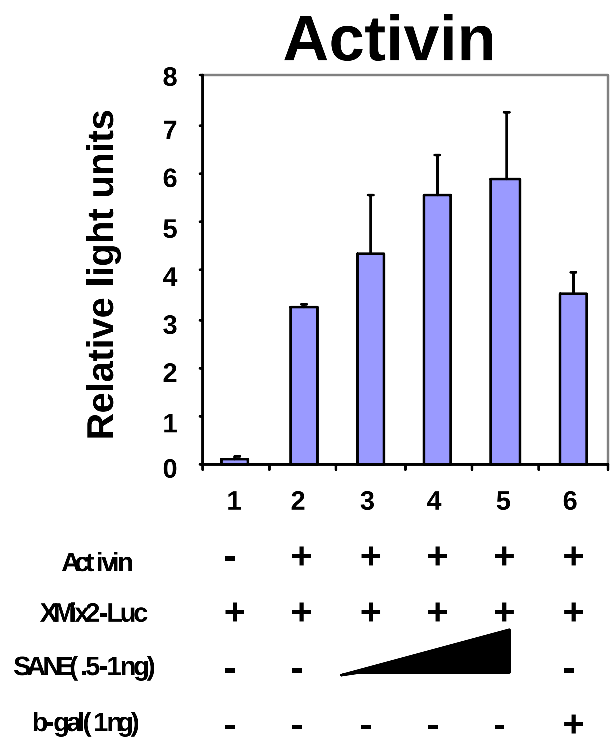


Transcription reporters for BMP and Activin/TGF-ß Signaling

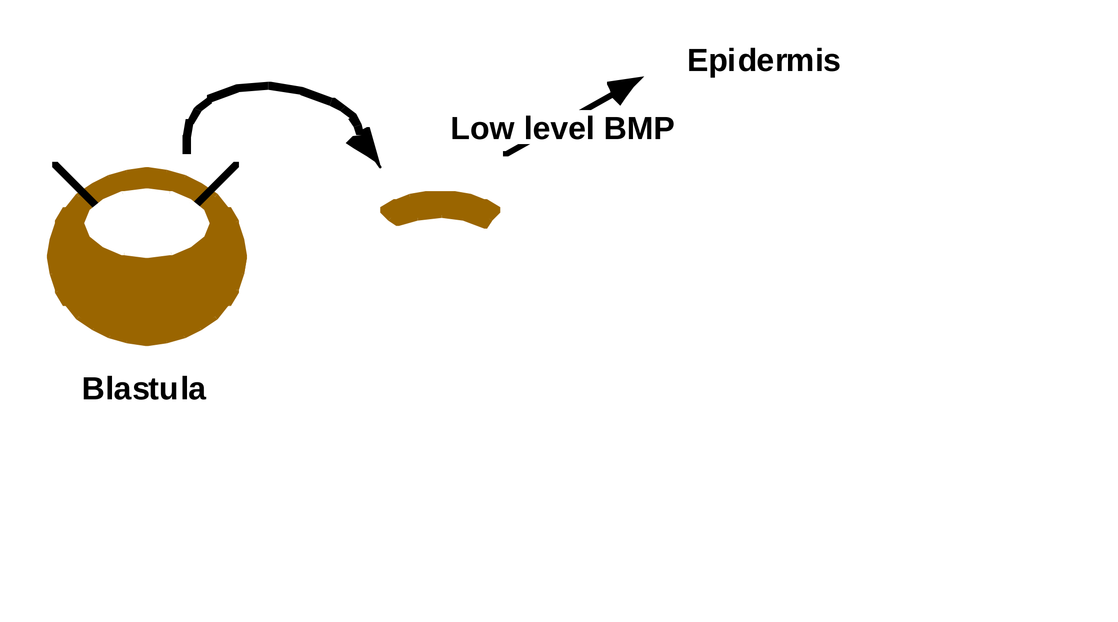


SANE/XMan1 inhibits BMP induced Xvent 2 transcription

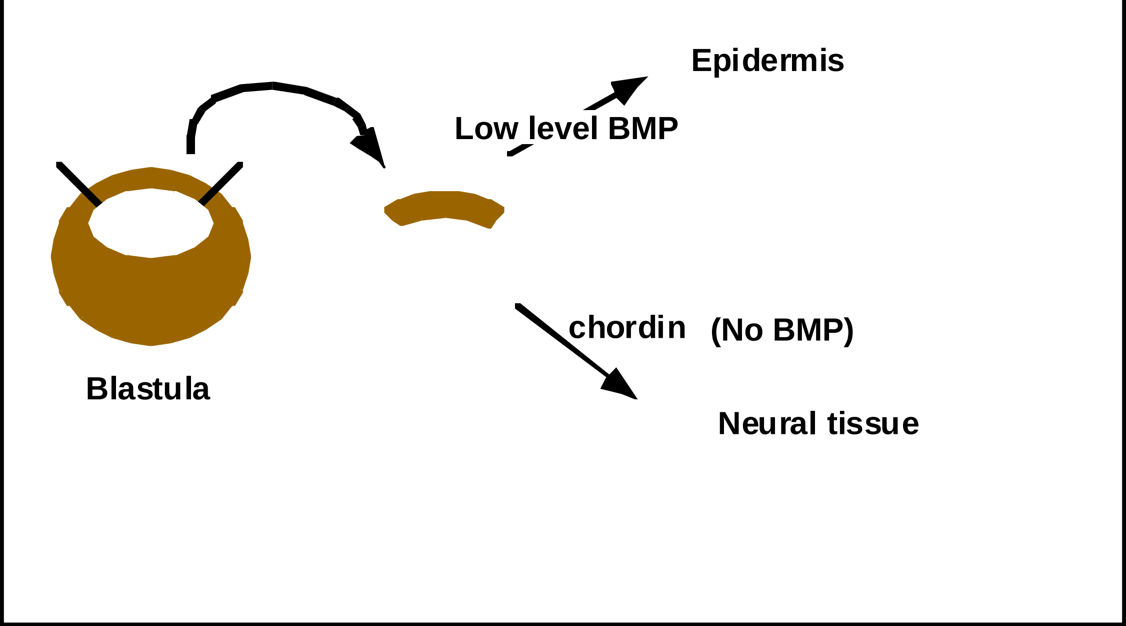




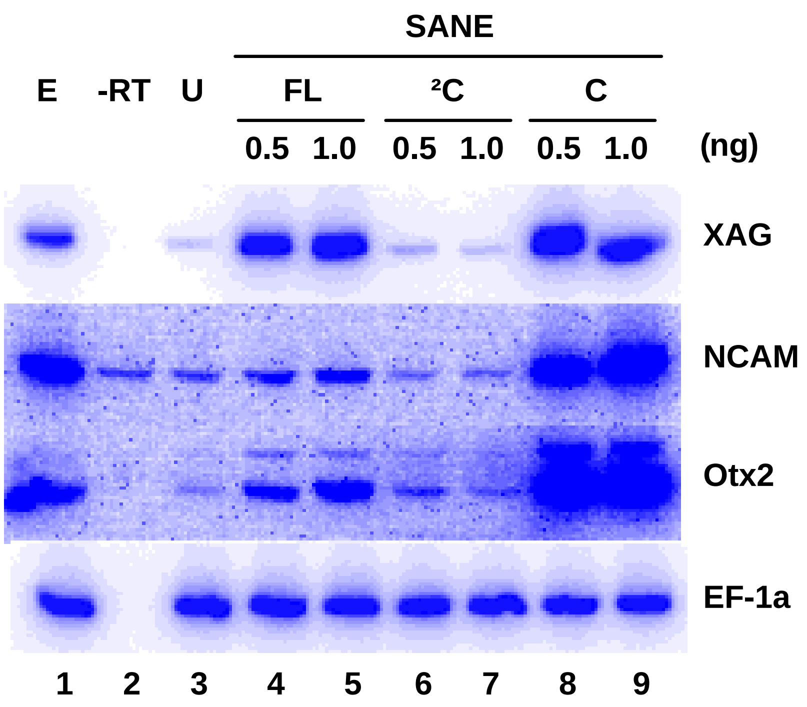
Neural induction in ectodermal explants (animal cap assay)



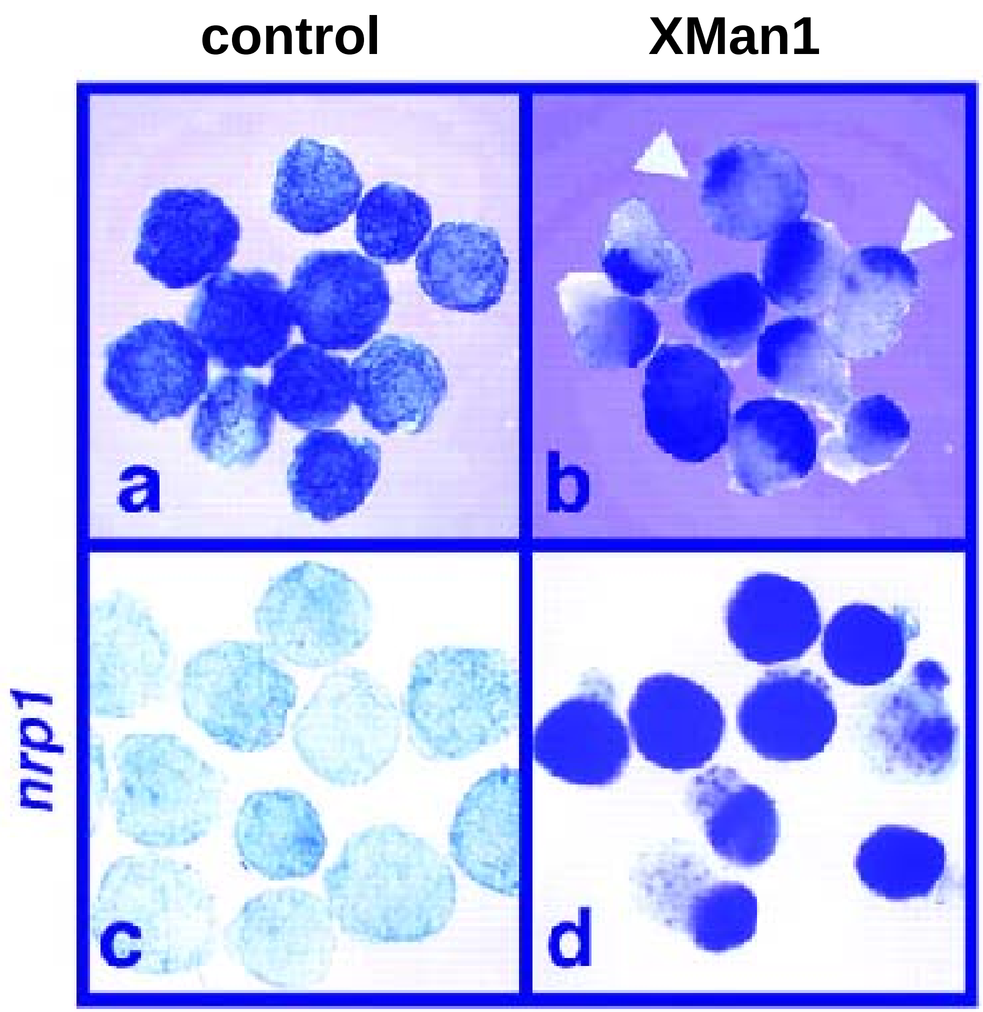
Neural induction in ectodermal explants (animal cap assay)



SANE/Xman1 is a neural inducer

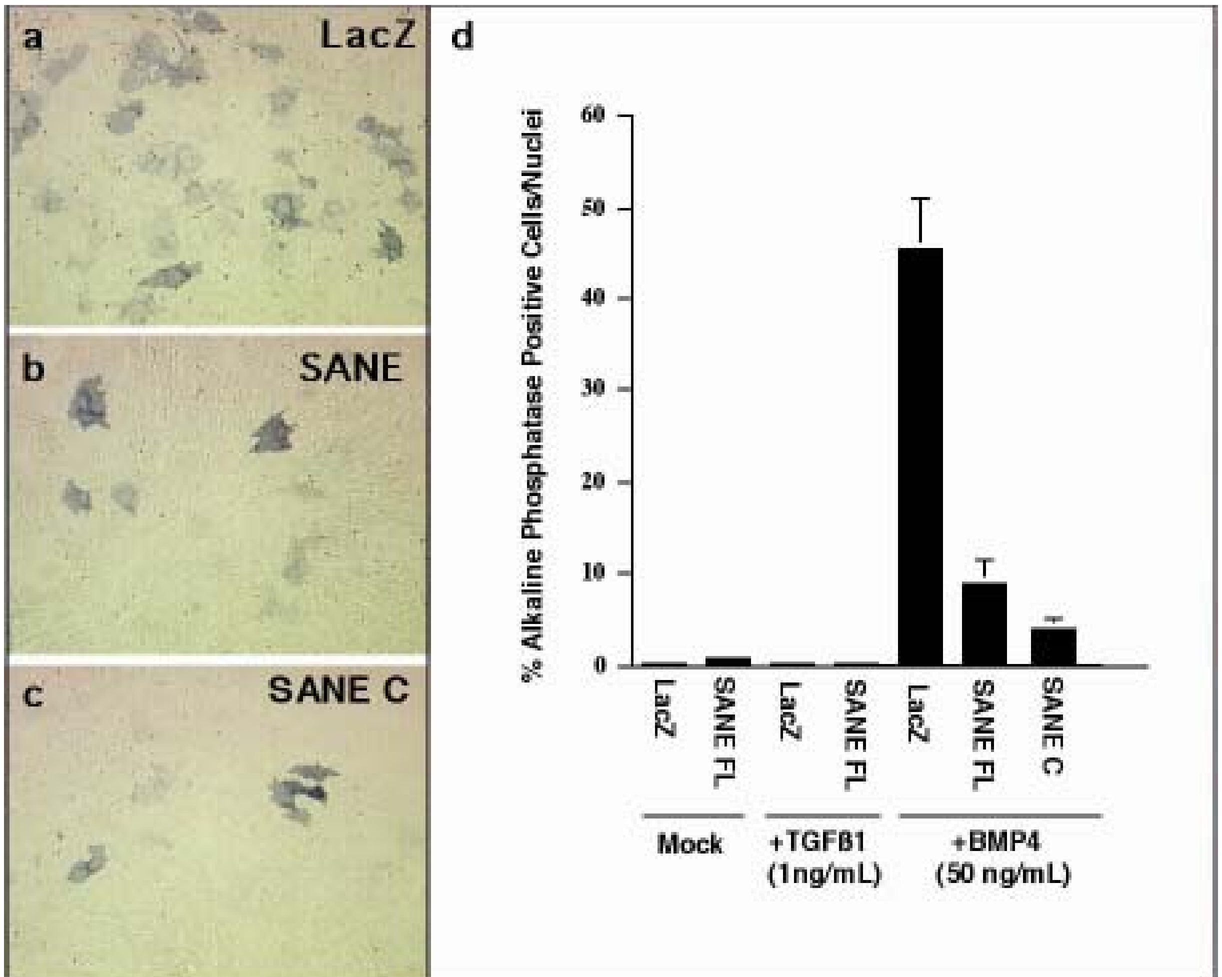


SANE/Xman1 is a neural inducer



Osada, S.-I. et al. Development 2003;130:1783-1794

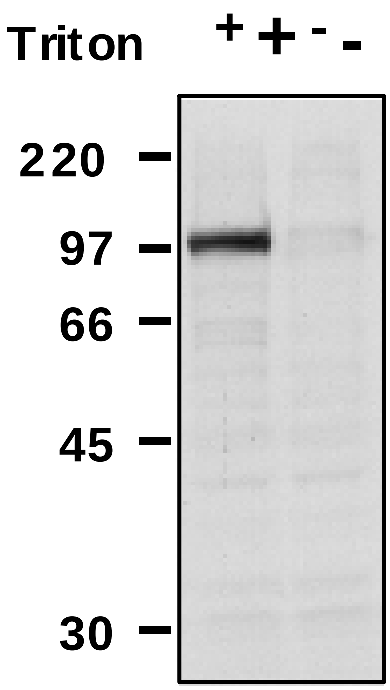
SANE/Man1 inhibits osteoblast formation induced by BMP



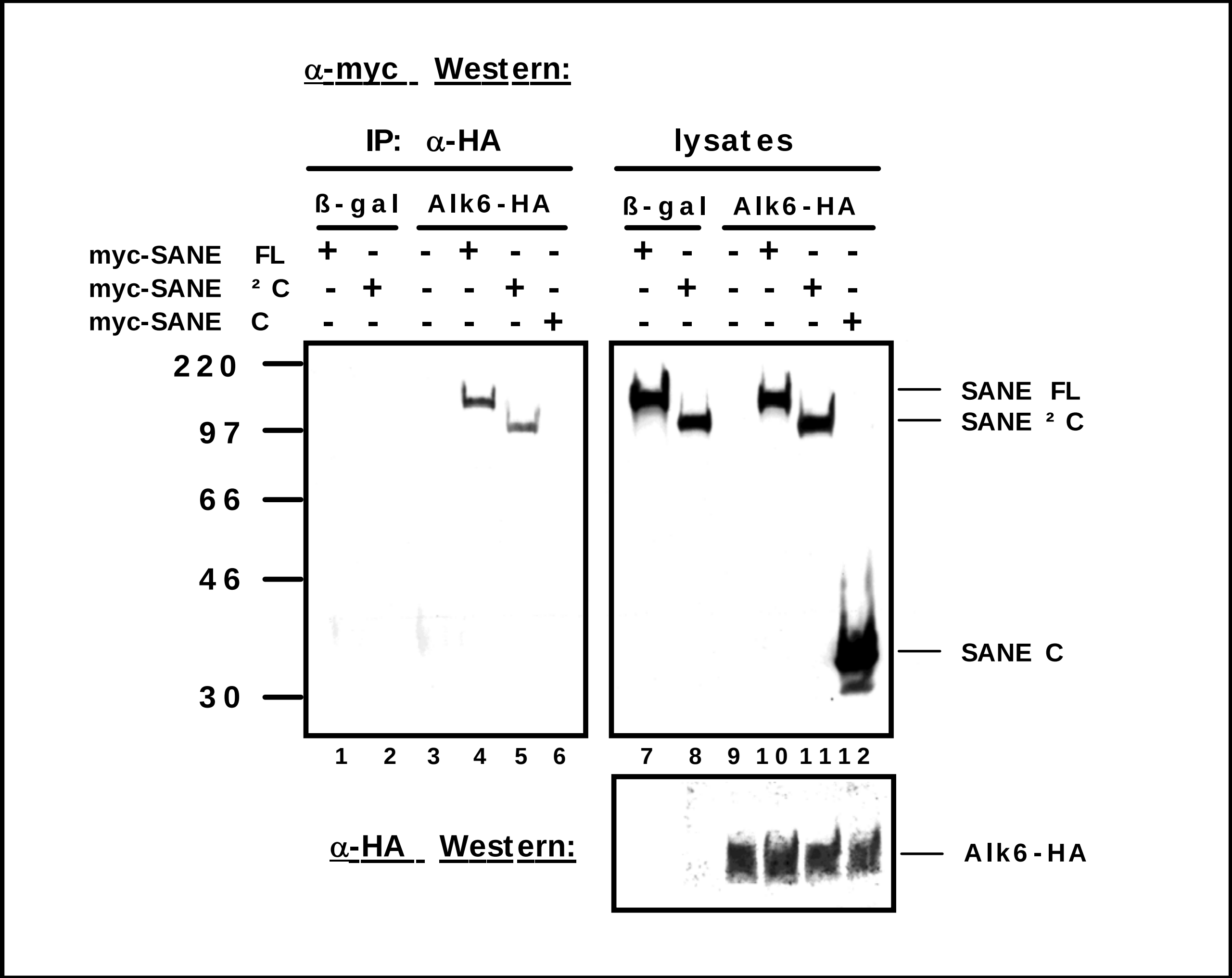
Summary:

- SANE/XMan1 binds BMP specific Smads (Smad1 and Smad5)
- SANE/XMan1 specifically inhibits BMP signaling

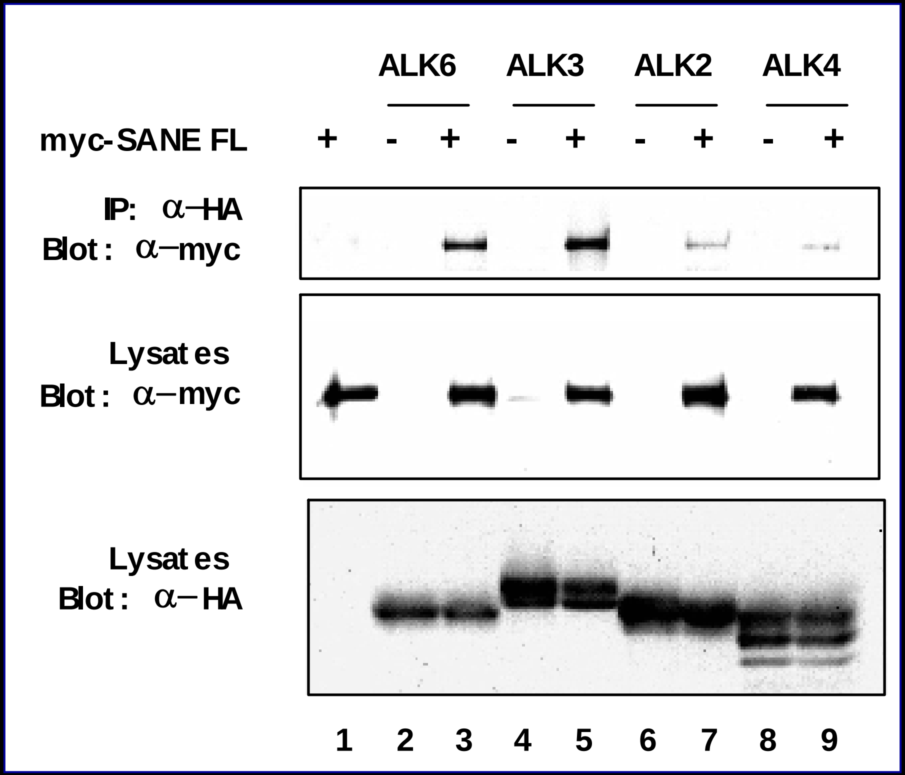
SANE/XMan1 is a membrane protein



SANE/XMan1 interacts with the BMP type I receptor



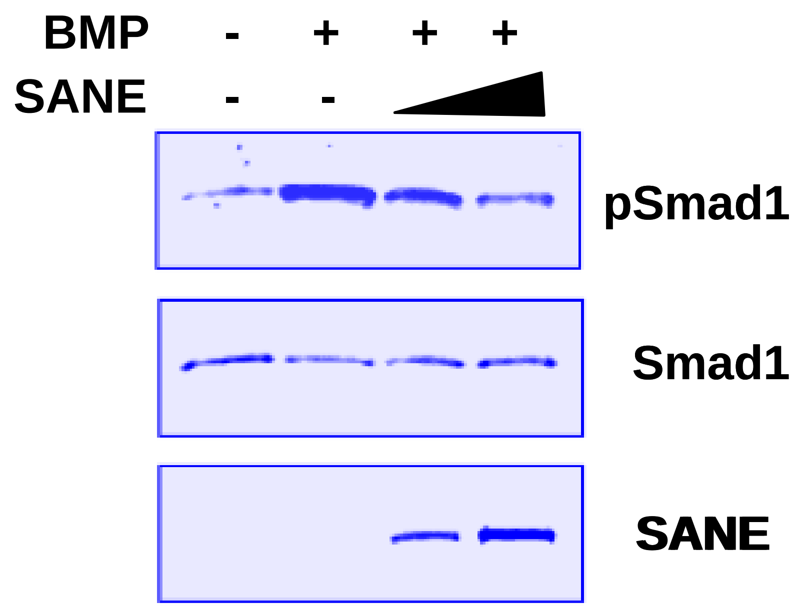
SANE/XMan1 interacts with the BMP type I receptor



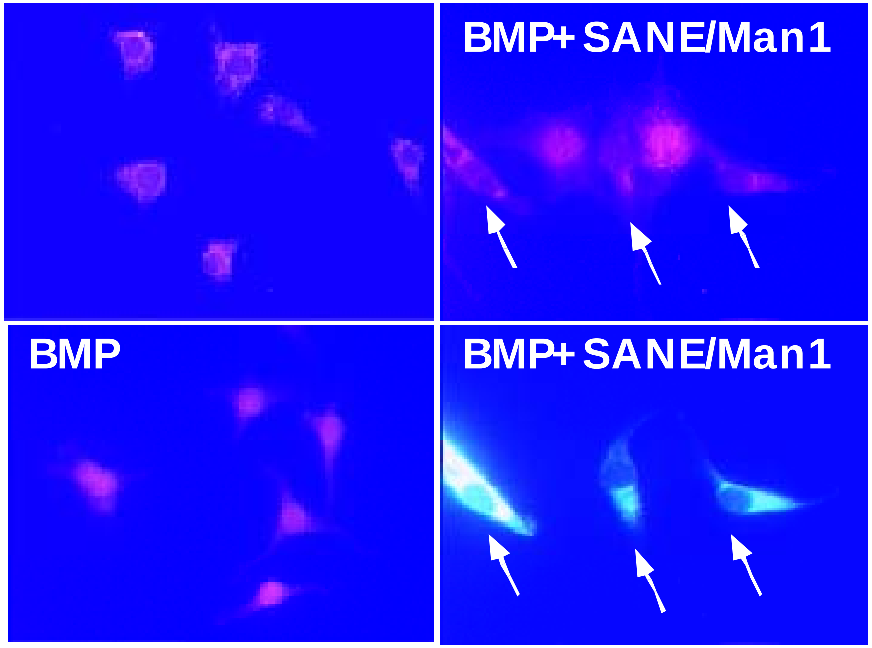
Summary:

- SANE/XMan1 binds BMP specific Smads (Smad1 and Smad5)
- SANE/XMan1 specifically inhibits BMP signaling
- SANE also binds to BMP receptors

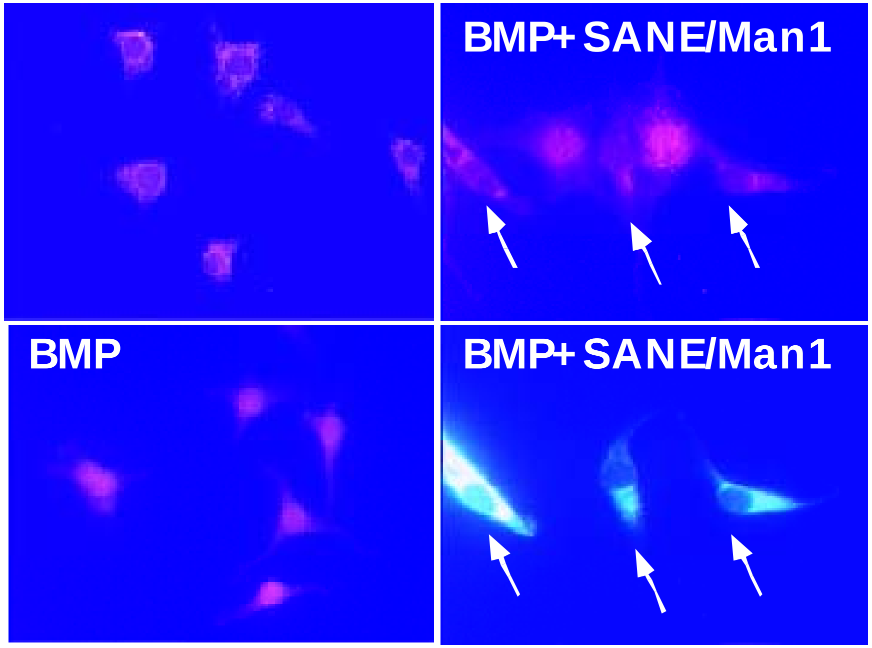
SANE/XMan1 blocks Smad1 phosphorylation

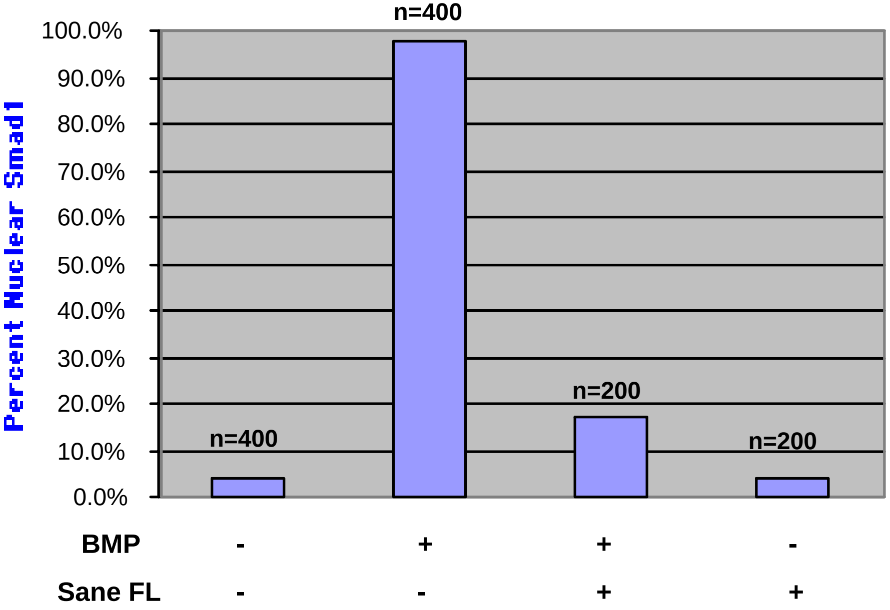


SANE/XMan1 blocks nuclear translocation of Smad1



SANE/XMan1 blocks nuclear translocation of Smad1

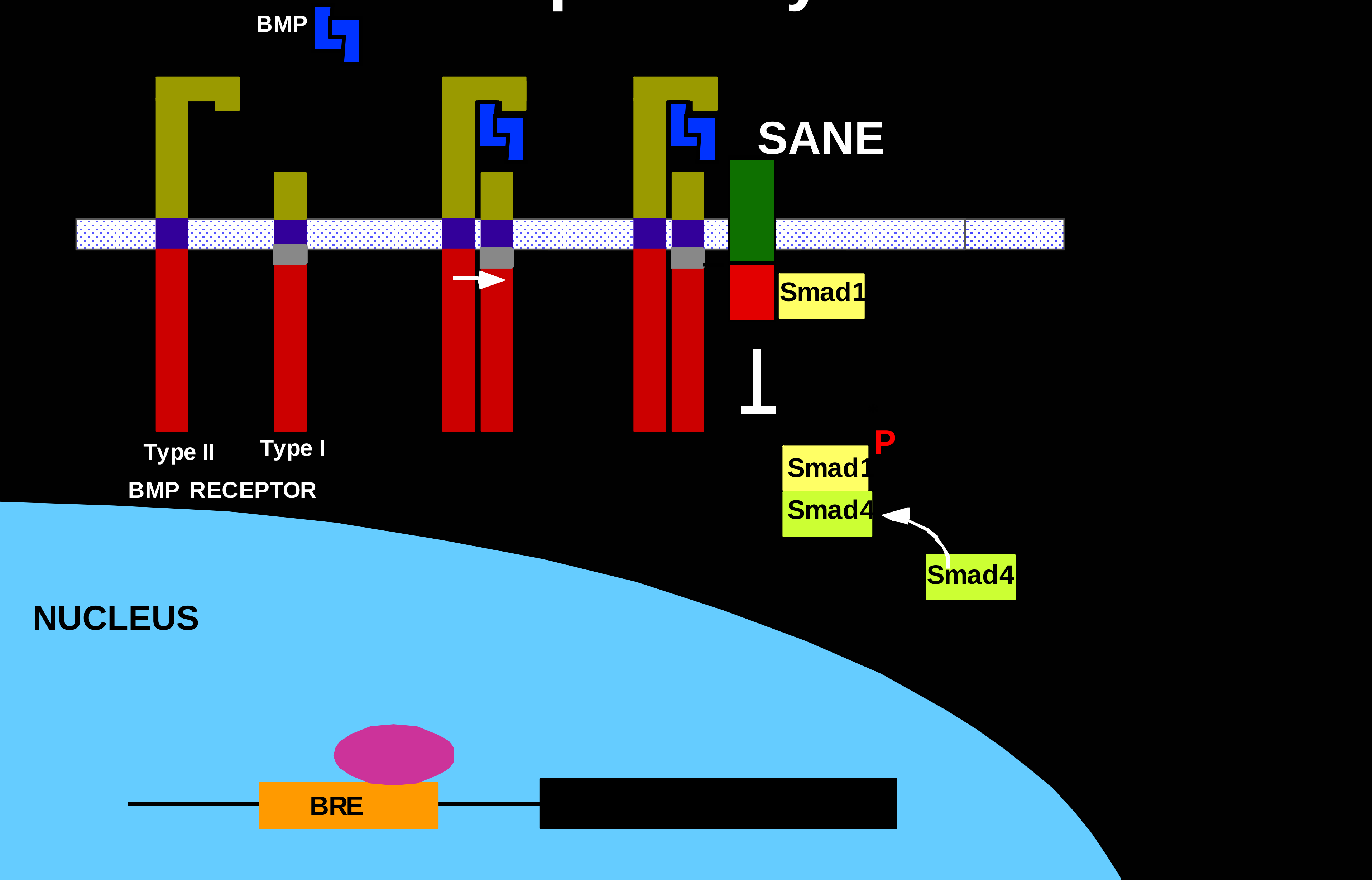




Summary:

- SANE/XMan1 binds BMP specific Smads (Smad1 and Smad5)
- SANE specifically inhibits BMP signaling
- SANE also binds to BMP receptors
- SANE inhibits Smad1 phosphorylation and nuclear translocation

Model for SANE function in BMP pathway



Acknowledgements

Hui-Chuan Huang

Peter Klein

G. Praveen Raju

Neviana Dimova

Collaboration:

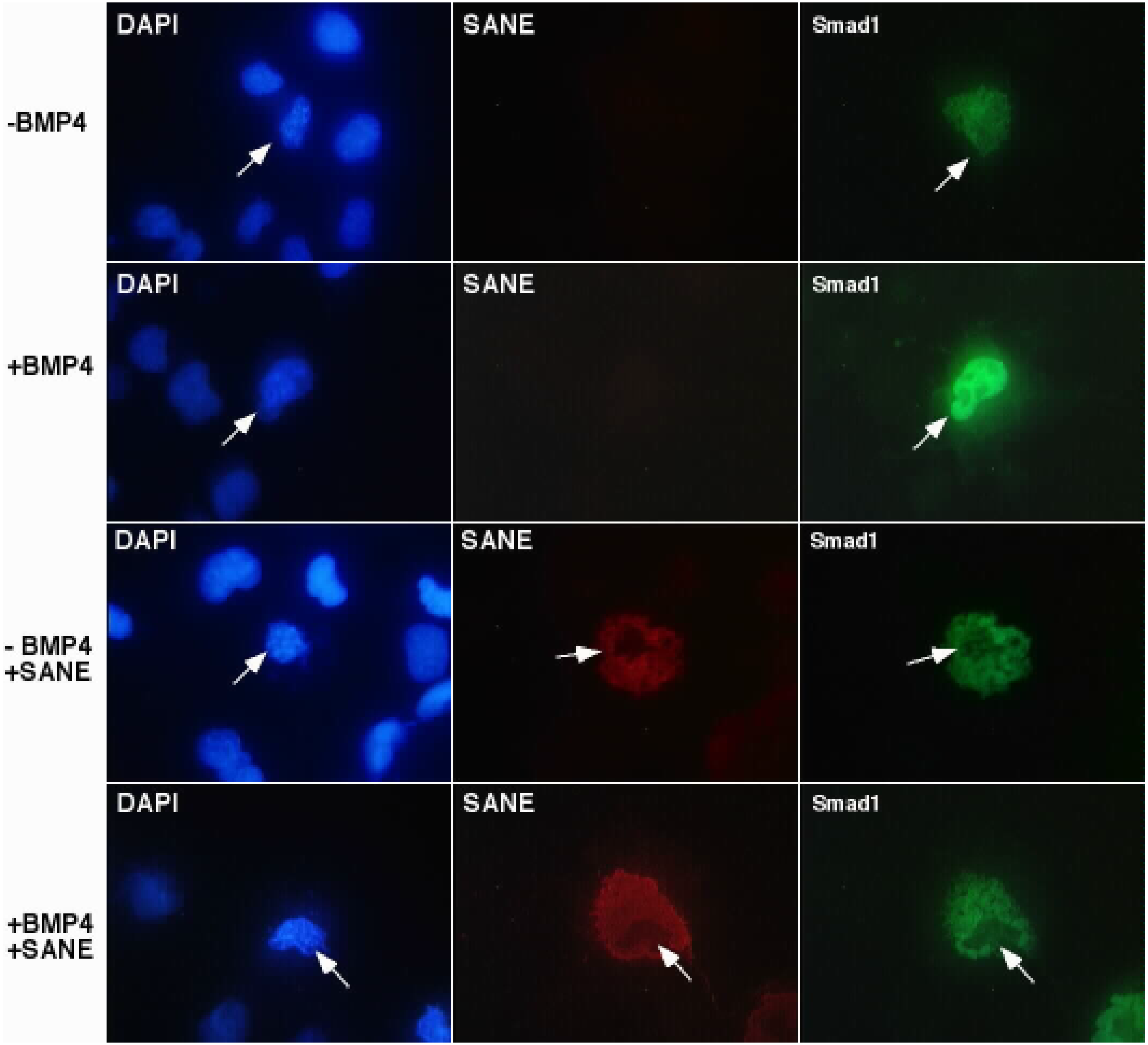
Frederick Kaplan

Eileen Shore

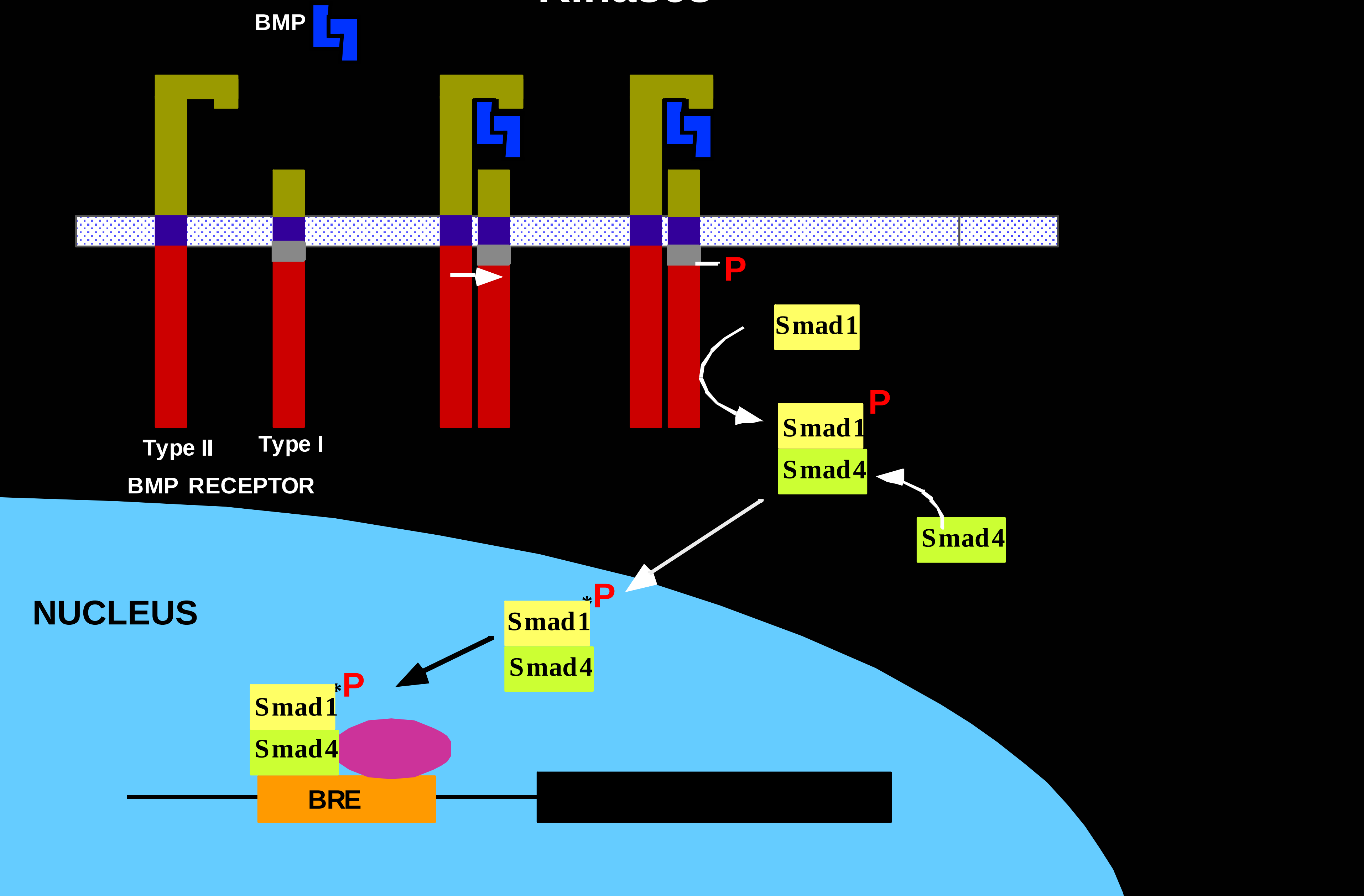
Funding (to HCH):

- NIH
- The Cali endowment for molecular studies in Fibrodysplasia ossificans progressiva (FOP)

SANE blocks nuclear translocation of Smad1



BMPs Signal through Receptor Serine/ Threonine Kinases



BMPs Signal through Receptor Serine/ Threonine Kinases