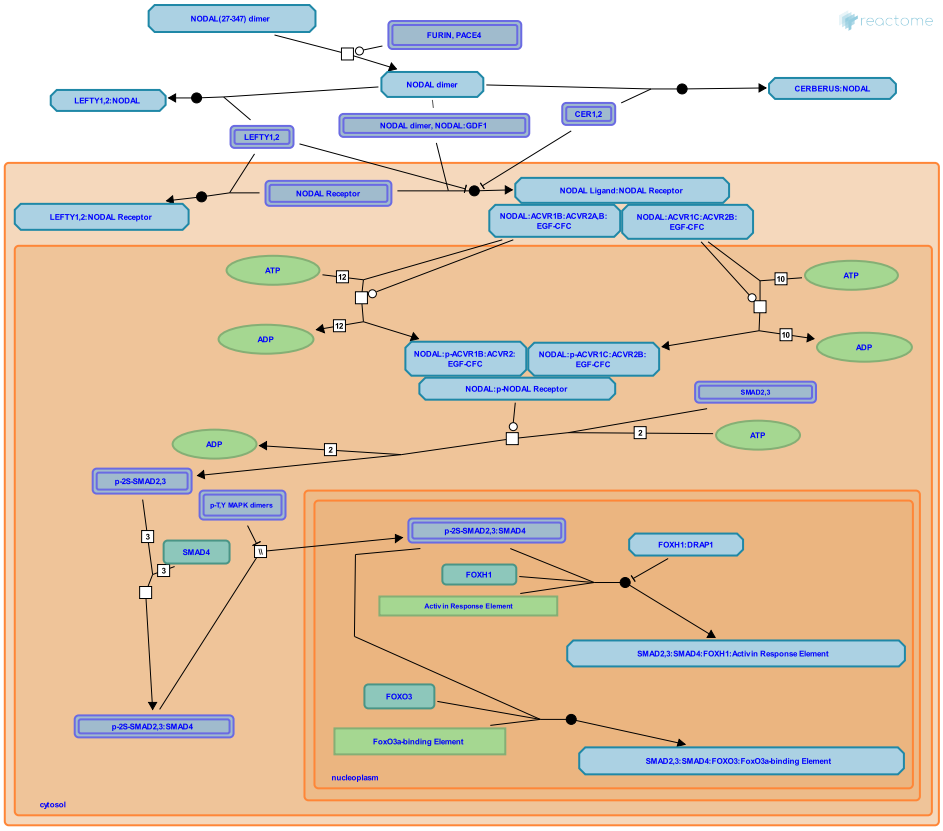
# Signaling by NODAL




Chen, YG., Contreras, O., Heldin, CH., Huang, T., Huminiecki, L., Jassal, B., May, B., Moustakas, A., Orlic-Milacic, M., Peng, C.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.



This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the Reactome Textbook.

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# Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.
The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

## Literature references

Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics, 18*, 142. ↗

Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics, 33*, 3461-3467. ↗

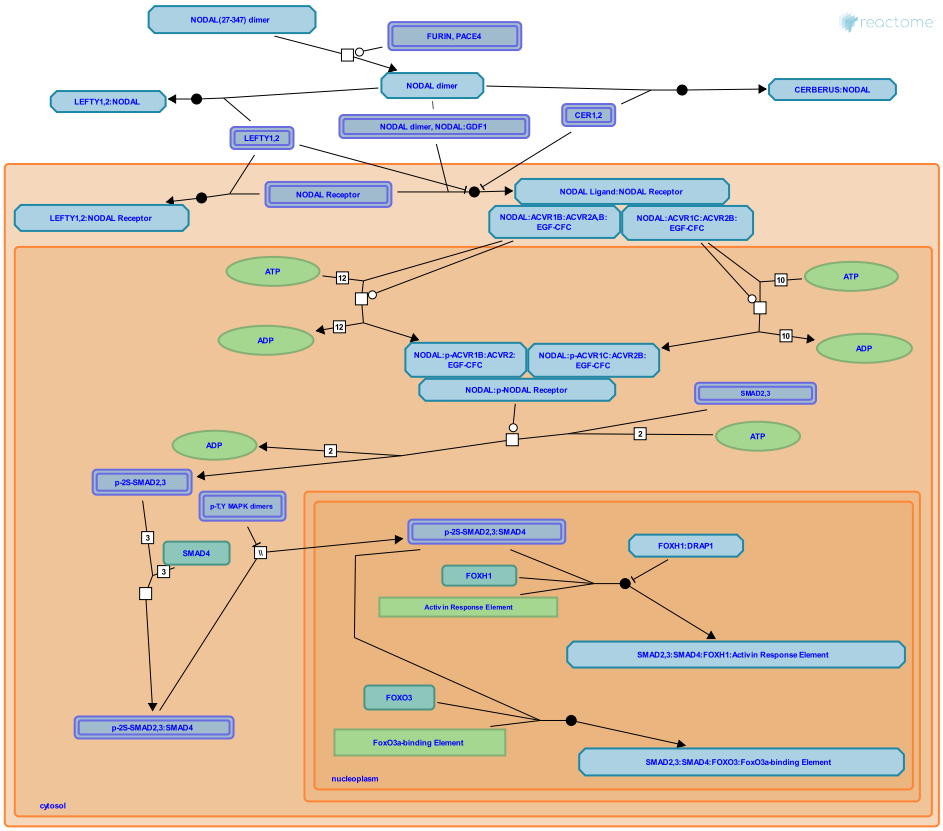
Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res, 46*, D649-D655. ↗

Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology, 14*, e1005968. ↗

## Signaling by NODAL ↗

**Stable identifier:** R-HSA-1181150

**Compartments:** cytosol, extracellular region, nucleoplasm, plasma membrane




Signaling by NODAL is essential for patterning of the axes of the embryo and formation of mesoderm and endoderm (reviewed in Schier 2009, Shen 2007). The NODAL proprotein is secreted and cleaved extracellularly to yield mature NODAL. Mature NODAL homodimerizes and can also form heterodimers with LEFTY1, LEFTY2, or CERBERUS, which negatively regulate NODAL signaling. NODAL also forms heterodimers with GDF1, which increases NODAL activity. NODAL dimers bind the NODAL receptor comprising a type I Activin receptor (ACVR1B or ACVR1C), a type II Activin receptor (ACVR2A or ACVR2B), and an EGF-CFC coreceptor (CRIPTO or CRYPTIC). After binding NODAL, the type II activin receptor phosphorylates the type I activin receptor which then phosphorylates SMAD2 and SMAD3 (R-SMADs). Phosphorylated SMAD2 and SMAD3 form hetero-oligomeric complexes with SMAD4 (CO-SMAD) and transit from the cytosol to the nucleus. Within the nucleus the SMAD complexes interact with transcription factors such as FOXH1 to activate transcription of target genes.

## Literature references


Shen, MM. (2007). Nodal signaling: developmental roles and regulation. *Development, 134*, 1023-34. ↗

Schier, AF. (2009). Nodal morphogens. *Cold Spring Harb Perspect Biol, 1*, a003459. ↗


## Editions

| | | |
|---|---|---|
| 2011-01-23 | Authored, Edited | May, B. |
| 2011-08-25 | Reviewed | Peng, C. |

## Cleavage of NODAL proprotein ↗

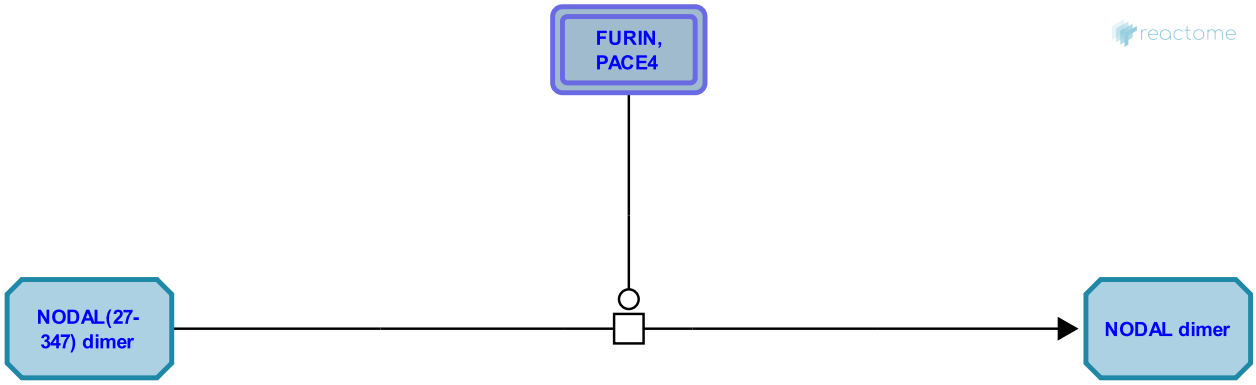
**Location:** Signaling by NODAL

**Stable identifier:** R-HSA-1181152

**Type:** transition

**Compartments:** extracellular region

**Inferred from:** Cleavage of Nodal proprotein (Mus musculus)




Either FURIN or PACE4 endoproteases cleave the 321 amino acid NODAL proprotein to yield the 110 amino acid NODAL mature protein. In cultured mouse cells the CRIPTO coreceptor at the plasma membrane recruits both NODAL proprotein and FURIN or PACE4 endoprotease.

**Followed by:** The NODAL Receptor binds NODAL ligands

## Editions

| | | |
|---|---|---|
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| 2011-08-25 | Reviewed | Peng, C. |

## The NODAL Receptor binds NODAL ligands ↗

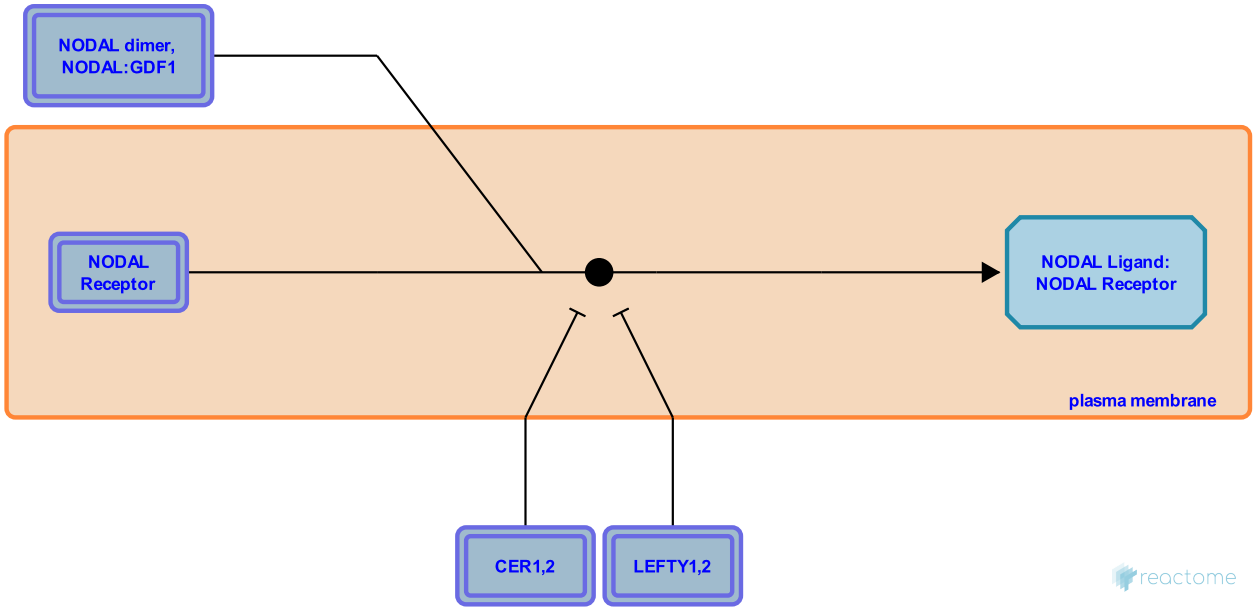
**Location:** Signaling by NODAL

**Stable identifier:** R-HSA-1181155

**Type:** binding

**Compartments:** plasma membrane, extracellular region

**Inferred from:** The Nodal Receptor binds Nodal ligands (Mus musculus)




NODAL binds a receptor comprising a type I activin receptor (ACVR1B or ACVR1C), a type II activin receptor (ACVR2 or ACVR2B), and a EGF-CFC coreceptor (CRIPTO or CRYPTIC). Though NODAL is able to signal via the ACVR1C (ALK7) receptor (Reissman et al. 2001), experiments in mouse indicate NODAL signaling via ALK7 is dispensable during embryogenesis (Jornvall et al. 2004).
CERBERUS (CER1) and probably DAND5 (CER2, inferred from mouse homologs) forms heterodimers with NODAL, preventing NODAL from activating the NODAL receptor.
LEFTY1 and LEFTY2 bind the EGF-CFC coreceptor (CRIPTO or CRYPTIC) and prevent it from interacting with Activin type I and type II receptors, thereby interfering with the assembly of the NODAL receptor.

**Preceded by:** Cleavage of NODAL proprotein

**Followed by:** Type II Activin Receptor (ActRII/ACVR2) phosphorylates Type I Activin Receptor (ActRIB/ACVR1B) in response to NODAL, Type II Activin Receptor (ActRIIB/ACVR2B) phosphorylates Type I Activin Receptor (ActRIC/ACVR1C) in response to NODAL

## Literature references


Vo, BT., Khan, SA. (2011). Expression of nodal and nodal receptors in prostate stem cells and prostate cancer cells: Autocrine effects on cell proliferation and migration. *Prostate.* ↗

Caniggia, I., Lye, S., Dunk, C., Peng, C., Munir, S., Nadeem, L. et al. (2011). Nodal Signals through Activin Receptor-Like Kinase 7 to Inhibit Trophoblast Migration and Invasion Implication in the Pathogenesis of Preeclampsia. *Am J Pathol, 178*, 1177-89. ↗

Tsang, BK., Peng, C., Munir, S., Zhong, Y., Yang, BB., Xu, G. (2004). Nodal induces apoptosis and inhibits proliferation in human epithelial ovarian cancer cells via activin receptor-like kinase 7. *J Clin Endocrinol Metab, 89*, 5523-34. ↗

Yang, B., Wu, Y., Peng, C., Lala, PK., Munir, S., Xu, G. (2004). Nodal and ALK7 inhibit proliferation and induce apoptosis in human trophoblast cells. *J Biol Chem, 279*, 31277-86. ↗

Jörnvall, H., Andersson, O., Reissmann, E., Mehrkash, M., Ibáñez, CF. (2004). ALK7, a receptor for nodal, is dispensable for embryogenesis and left-right patterning in the mouse. *Mol Cell Biol, 24,* 9383-9. ↗

## Editions

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|---|---|---|
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## Type II Activin Receptor (ActRII/ACVR2) phosphorylates Type I Activin Receptor (ActRIB/ACVR1B) in response to NODAL ↗

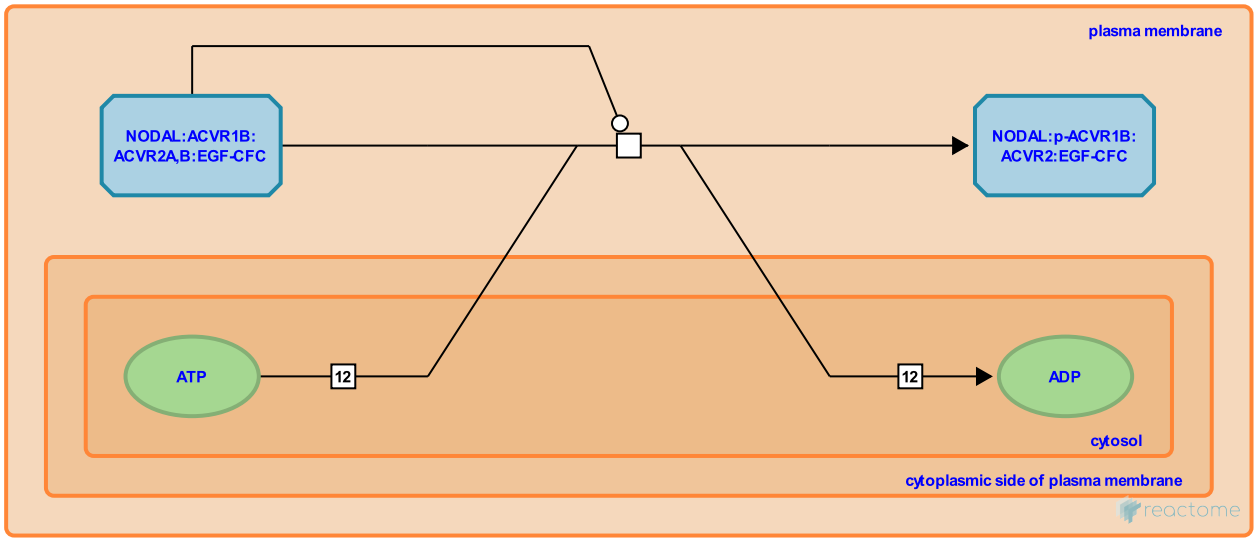
**Location:** Signaling by NODAL

**Stable identifier:** R-HSA-1181156

**Type:** transition

**Compartments:** plasma membrane, cytosol

**Inferred from:** ACVR2A,B (ActRIIA,B) phosphorylates ACVR1B (ActRIB, ALK4) in response to Activin (Homo sapiens)




As inferred from the response of the activin receptor to activin, the type II component of the NODAL receptor phosphorylates the type I component in response to NODAL binding. Experiments with human proteins in frog oocytes show NODAL can signal via the CRIPTO:ACVR1B(ALK4):ACVR2 complex (Yeo and Whitman 2001).

**Preceded by:** The NODAL Receptor binds NODAL ligands

**Followed by:** Phosphorylation of R-SMAD2/3 by NODAL receptor

### Literature references

Yeo, C., Whitman, M. (2001). Nodal signals to Smads through Cripto-dependent and Cripto-independent mechanisms . *Mol Cell, 7*, 949-57. ↗

### Editions

| | | |
|---|---|---|
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## Type II Activin Receptor (ActRIIB/ACVR2B) phosphorylates Type I Activin Receptor (ActRIC/ACVR1C) in response to NODAL ↗

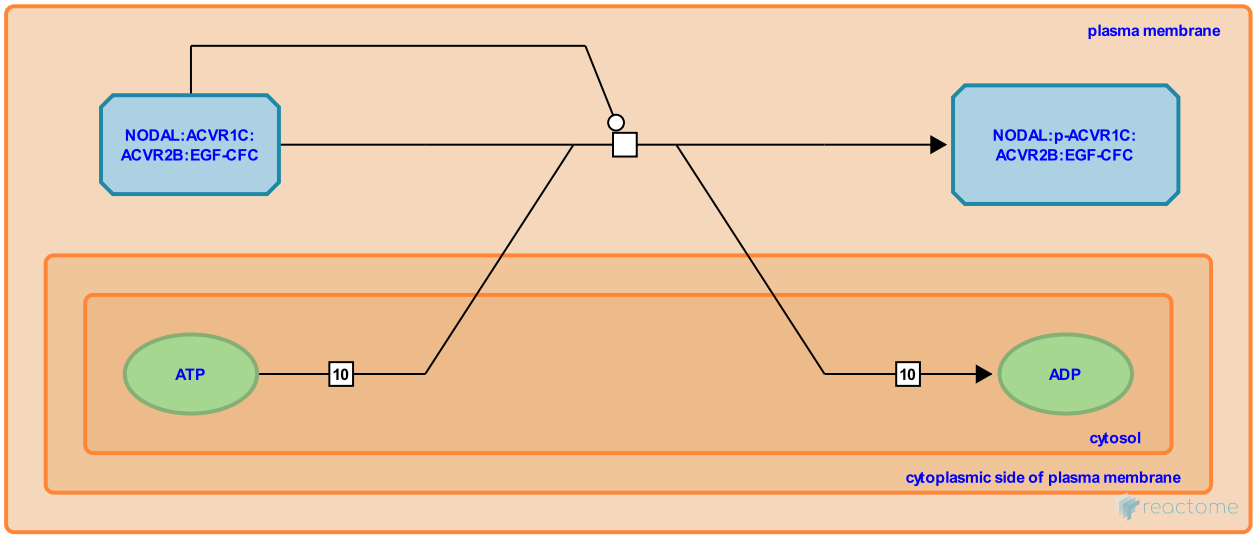
**Location:** Signaling by NODAL

**Stable identifier:** R-HSA-1225894

**Type:** transition

**Compartments:** plasma membrane, cytosol

**Inferred from:** ACVR2A,B (ActRIIA,B) phosphorylates ACVR1B (ActRIB, ALK4) in response to Activin (Homo sapiens)




As inferred from the response of the activin receptor to activin, the type II component of the NODAL receptor phosphorylates the type I component in response to NODAL binding. As inferred from mouse and frog (Xenopus) NODAL can signal via the ACVR1C (ALK7) type I activin receptor (Reissman et al. 2001) though this may be dispensable for development in mouse (Jornvall et al. 2004).

**Preceded by:** The NODAL Receptor binds NODAL ligands

**Followed by:** Phosphorylation of R-SMAD2/3 by NODAL receptor

### Literature references


Caniggia, I., Lye, S., Dunk, C., Peng, C., Munir, S., Nadeem, L. et al. (2011). Nodal Signals through Activin Receptor-Like Kinase 7 to Inhibit Trophoblast Migration and Invasion Implication in the Pathogenesis of Preeclampsia. *Am J Pathol, 178*, 1177-89. ↗

Peng, C., Ye, G., Lee, D., Zhong, Y., Modica-Amore, J., Xu, G. (2009). Nodal and activin receptor-like kinase 7 induce apoptosis in human breast cancer cell lines: Role of caspase 3. *Int J Physiol Pathophysiol Pharmacol, 1*, 83-96. ↗

Jörnvall, H., Andersson, O., Minchiotti, G., Blokzijl, A., Persico, MG., Reissmann, E. et al. (2001). The orphan receptor ALK7 and the Activin receptor ALK4 mediate signaling by Nodal proteins during vertebrate development. *Genes Dev, 15*, 2010-22. ↗

Jörnvall, H., Andersson, O., Reissmann, E., Mehrkash, M., Ibáñez, CF. (2004). ALK7, a receptor for nodal, is dispensable for embryogenesis and left-right patterning in the mouse. *Mol Cell Biol, 24*, 9383-9. ↗


### Editions

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## Phosphorylation of R-SMAD2/3 by NODAL receptor ↗

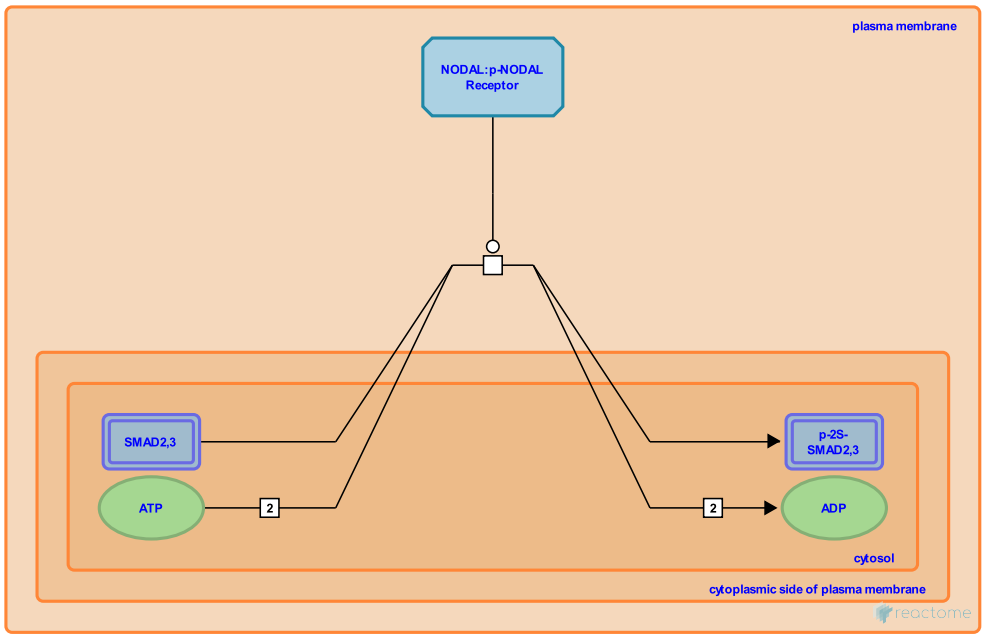
**Location:** Signaling by NODAL

**Stable identifier:** R-HSA-1181355

**Type:** transition

**Compartments:** plasma membrane, cytosol

**Inferred from:** Phosphorylation of Smad2 by Nodal Receptor (Homo sapiens)




NODAL receptors signal by phosphorylating SMAD2 and SMAD3 (Bondestam et al. 2001, Kumar et al. 2001, DaCosta Byfield et al. 2004). As in TGF-beta signaling, Smad anchor for receptor activation (SARA) may bind and present SMAD2 and SMAD3 for phosphorylation but this has not yet been demonstrated in NODAL signaling.

**Preceded by:** Type II Activin Receptor (ActRII/ACVR2) phosphorylates Type I Activin Receptor (ActRIB/ACVR1B) in response to NODAL, Type II Activin Receptor (ActRIIB/ACVR2B) phosphorylates Type I Activin Receptor (ActRIC/ACVR1C) in response to NODAL

**Followed by:** Phosphorylated SMAD2 and SMAD3 form a complex with SMAD4

### Literature references


DaCosta Byfield, S., Major, C., Roberts, AB., Laping, NJ. (2004). SB-505124 is a selective inhibitor of transforming growth factor-beta type I receptors ALK4, ALK5, and ALK7. *Mol Pharmacol, 65*, 744-52. ↗

Otonkoski, T., Kaivo-Oja, N., Aaltonen, J., Kallio, J., Ustinov, J., Horelli-Kuitunen, N. et al. (2001). cDNA cloning, expression studies and chromosome mapping of human type I serine/threonine kinase receptor ALK7 (ACVR1C). *Cytogenet Cell Genet, 95*, 157-62. ↗

Kuehn, MR., Celeste, AJ., ten Dijke, P., Wolfman, NM., Novoselov, V., Kumar, A. (2001). Nodal signaling uses activin and transforming growth factor-beta receptor-regulated Smads. *J Biol Chem, 276*, 656-61. ↗


### Editions

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|---|---|---|
| 2011-01-28 | Authored, Edited | May, B. |
| 2011-08-25 | Reviewed | Peng, C. |

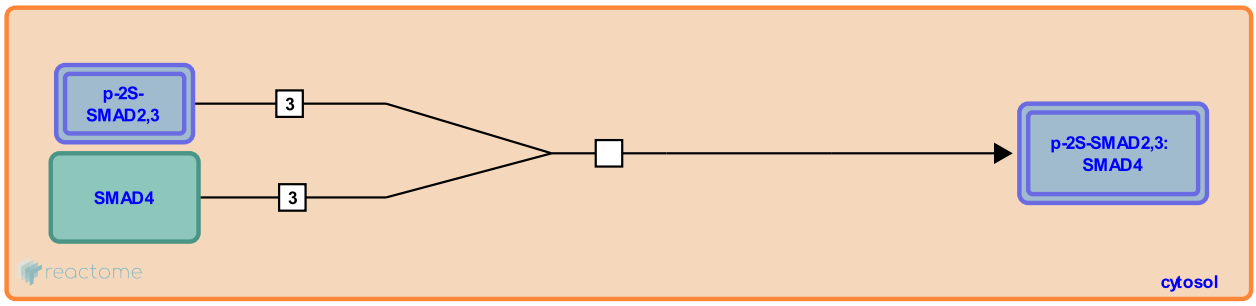
## Phosphorylated SMAD2 and SMAD3 form a complex with SMAD4 ↗

**Location:** Signaling by NODAL

**Stable identifier:** R-HSA-170847

**Type:** transition

**Compartments:** cytosol




The phosphorylated C-terminal tail of R-SMAD induces a conformational change in the MH2 domain (Qin et al. 2001, Chacko et al. 2004), which now acquires high affinity towards Co-SMAD i.e. SMAD4 (common mediator of signal transduction in TGF-beta/BMP signaling). The R-SMAD:Co-SMAD complex (Nakao et al. 1997) most likely is a trimer of two R-SMADs with one Co-SMAD (Kawabata et al. 1998). It is important to note that the Co-SMAD itself cannot be phosphorylated as it lacks the C-terminal serine motif.

ZFYVE16 (endofin) promotes SMAD heterotrimer formation. ZFYVE16 can bind TGFBR1 and facilitate SMAD2 phosphorylation, and it can also bind SMAD4, but the exact mechanism of ZFYVE16 (endofin) action in the context of TGF-beta receptor signaling is not known (Chen et al. 2007).

SARS-CoV-1 nucleocapsid protein (N) associates with SMAD3 and this binding interferes with the complex formation between SMAD3 and SMAD4. By this mechanism N modulates TGF-beta signaling to block apoptosis of SARS-CoV-infected host cells (Zhao et al. 2008).

**Preceded by:** Phosphorylation of R-SMAD2/3 by NODAL receptor

**Followed by:** The SMAD2/3:SMAD4 complex transfers to the nucleus

## Literature references


Lin, K., Correia, JJ., Chacko, BM., Lam, SS., Qin, BY., de Caestecker, MP. (2001). Structural basis of Smad1 activation by receptor kinase phosphorylation. *Mol Cell, 8*, 1303-12. ↗

Miyazono, K., Inoue, H., Kawabata, M., Imamura, T., Hanyu, A. (1998). Smad proteins exist as monomers in vivo and undergo homo- and hetero-oligomerization upon activation by serine/threonine kinase receptors. *EMBO J, 17*, 4056-65. ↗

Wang, Z., Chen, YG., Lu, Z., Zhang, L., Ma, J. (2007). Endofin, a FYVE domain protein, interacts with Smad4 and facilitates transforming growth factor-beta signaling. *J. Biol. Chem., 282*, 9688-95. ↗

Shi, G., De Caestecker, M., Lin, K., Chacko, BM., Hayward, LJ., Tiwari, A. et al. (2004). Structural basis of heteromeric smad protein assembly in TGF-beta signaling. *Mol Cell, 15*, 813-23. ↗

Kyin, S., Rigotti, DJ., Shi, Y., Massague, J., Muir, TW., Li, C. et al. (2001). Crystal structure of a phosphorylated Smad2. Recognition of phosphoserine by the MH2 domain and insights on Smad function in TGF-beta signaling. *Mol Cell, 8*, 1277-89. ↗

## Editions

| | | |
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| 2006-01-18 | Edited | Jassal, B. |
| 2006-02-02 | Authored | Jassal, B., Heldin, CH., Moustakas, A., Huminiecki, L. |
| 2006-04-18 | Reviewed | Heldin, CH. |
| 2012-04-04 | Revised | Orlic-Milacic, M. |
| 2012-04-10 | Edited | Jassal, B. |
| 2012-05-14 | Reviewed | Huang, T. |
| 2012-11-14 | Reviewed | Chen, YG. |
| 2013-02-05 | Revised | Orlic-Milacic, M. |
| 2022-05-02 | Reviewed | Contreras, O. |
| 2022-05-09 | Edited | Orlic-Milacic, M. |

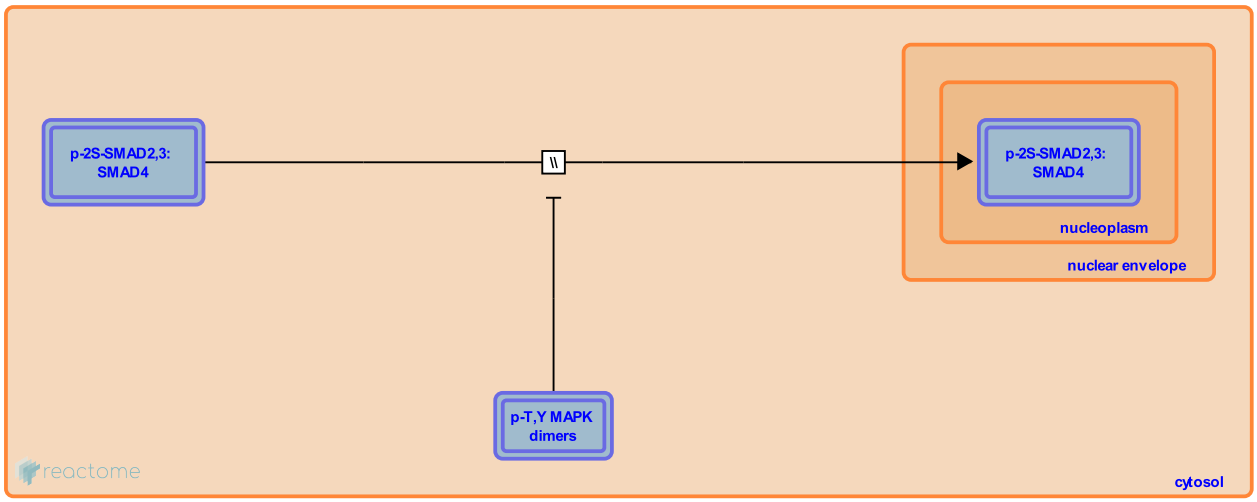
## The SMAD2/3:SMAD4 complex transfers to the nucleus ↗

**Location:** Signaling by NODAL

**Stable identifier:** R-HSA-173488

**Type:** omitted

**Compartments:** cytosol, nucleoplasm




The phosphorylated R-SMAD:CO-SMAD complex rapidly translocates to the nucleus (Xu et al. 2000, Kurisaki et al. 2001, Xiao et al. 2003) where it binds directly to DNA and interacts with a plethora of transcription co-factors. Translocation of SMAD2 and SMAD3 to the nucleus is negatively regulated by ERK-mediated phosphorylation (Kretzschmar et al. 1999). Regulation of target gene expression can be either positive or negative. A classic example of a target gene of the pathway are the genes encoding for I-SMADs. Thus, TGF-beta/SMAD signaling induces the expression of the negative regulators of the pathway (negative feedback loop).

**Preceded by:** Phosphorylated SMAD2 and SMAD3 form a complex with SMAD4

**Followed by:** Phospho R-SMAD(SMAD2,3):CO-SMAD(SMAD4):FOXH1 binds Activin Response Element, Phospho R-SMAD(SMAD2/3):CO-SMAD(SMAD4):FOXO3 binds FoxO3a-binding elements

## Literature references


Yoneda, Y., Moustakas, A., Heldin, CH., Kose, S., Kurisaki, A. (2001). Transforming growth factor-beta induces nuclear import of Smad3 in an importin-beta1 and Ran-dependent manner. *Mol Biol Cell, 12*, 1079-91. ↗

Lin, X., Feng, XH., Duan, X., Liang, YY., Dai, F. (2010). Coupling of dephosphorylation and nuclear export of Smads in TGF-beta signaling. *Methods Mol. Biol., 647*, 125-37. ↗

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## Editions

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|---|---|---|
| 2006-02-02 | Authored | Jassal, B., Heldin, CH., Moustakas, A., Huminiecki, L. |
| 2006-02-10 | Edited | Jassal, B. |
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| 2012-11-14 | Reviewed | Chen, YG. |
| 2022-05-02 | Reviewed | Contreras, O. |
| 2022-05-09 | Edited | Orlic-Milacic, M. |

## Phospho R-SMAD(SMAD2,3):CO-SMAD(SMAD4):FOXH1 binds Activin Response Element ↗

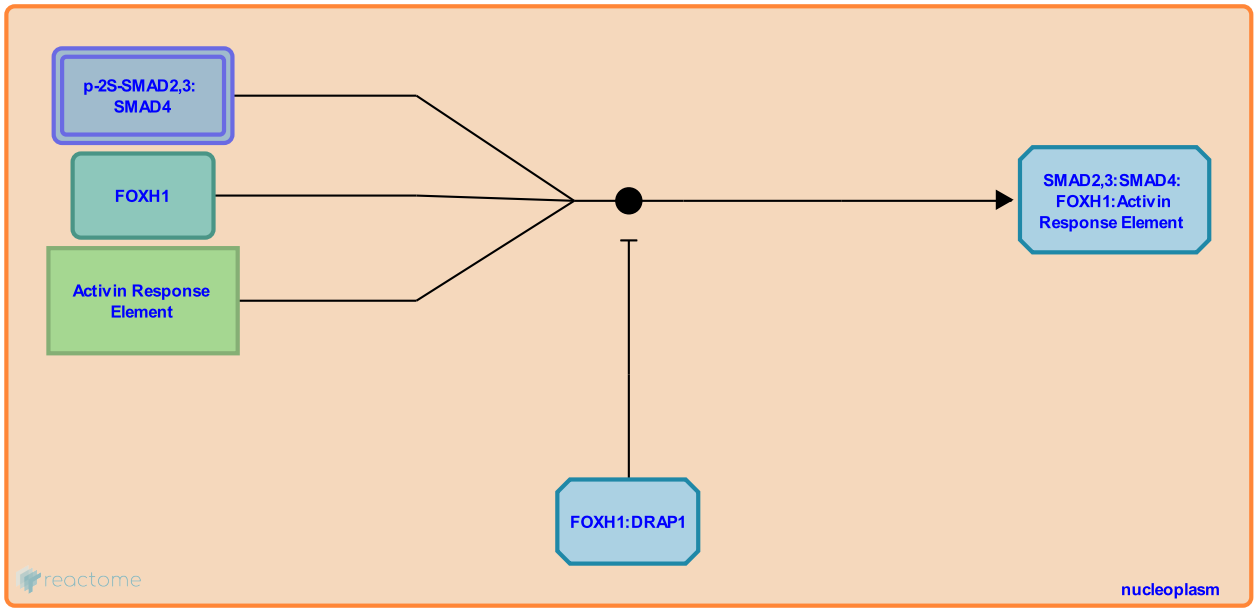
**Location:** Signaling by NODAL

**Stable identifier:** R-HSA-1225919

**Type:** binding

**Compartments:** nucleoplasm

**Inferred from:** p-Smad2,3:Smad4:Foxh1 binds Activin Response Element (Mus musculus)




As inferred from mouse, DRAP1 binds FOXH1 and inhibits activation of gene expression in response to NODAL signaling.
SMAD2 and SMAD3 do not bind DNA efficiently. They must interact with DNA-binding proteins to activate transcription. FOXH1 interacts with phospho-SMAD2 and phospho-SMAD3 complexed with CO-SMAD (SMAD4) at promoters containing the Activin Response Element (Zhou et al. 1998, Yanagisawa et al. 2000, inferred from Xenopus in Chen et al. 1996, Chen et al. 1997, Yeo et al. 1999). Follicle-stimulating hormone beta subunit (FSHB) and the Lim1 homeobox gene (LXH1) are examples of genes regulated by Activin.

**Preceded by:** The SMAD2/3:SMAD4 complex transfers to the nucleus

## Literature references


Yeo, CY., Chen, X., Whitman, M. (1999). The role of FAST-1 and Smads in transcriptional regulation by activin during early Xenopus embryogenesis. *J. Biol. Chem., 274*, 26584-90. ↗

Weisberg, E., Watanabe, M., Fridmacher, V., Naco, G., Chen, X., Whitman, M. (1997). Smad4 and FAST-1 in the assembly of activin-responsive factor. *Nature, 389*, 85-9. ↗

Sugiyama, M., Uchida, K., Takahashi, T., Masuda, A., Yamaki, K., Saito, T. et al. (2000). Heterogeneities in the biological and biochemical functions of Smad2 and Smad4 mutants naturally occurring in human lung cancers. *Oncogene, 19*, 2305-11. ↗

Zawel, L., Zhou, S., Kinzler, KW., Vogelstein, B., Lengauer, C. (1998). Characterization of human FAST-1, a TGF beta and activin signal transducer. *Mol Cell, 2*, 121-7. ↗

Rubock, MJ., Chen, X., Whitman, M. (1996). A transcriptional partner for MAD proteins in TGF-beta signalling. *Nature, 383*, 691-6. ↗

## Editions

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|---|---|---|
| 2011-02-14 | Authored, Edited | May, B. |
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| 2012-11-14 | Reviewed | Chen, YG. |

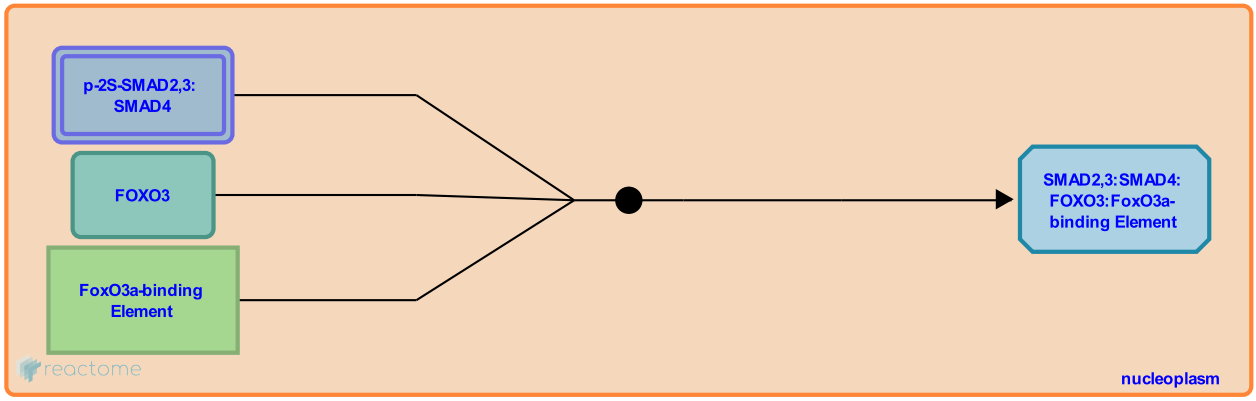
## Phospho R-SMAD(SMAD2/3):CO-SMAD(SMAD4):FOXO3 binds FoxO3a-binding elements ↗

**Location:** Signaling by NODAL

**Stable identifier:** R-HSA-1535903

**Type:** binding

**Compartments:** nucleoplasm




FOXO3 (FOXO3A) interacts with phospho-SMAD2 and phospho-SMAD3 complexed with CO-SMAD (SMAD4) at a promoter containing the FoxO3a-binding Element (Fu and Peng 20110).

**Preceded by:** The SMAD2/3:SMAD4 complex transfers to the nucleus

### Literature references

Peng, C., Fu, G. (2011). Nodal enhances the activity of FoxO3a and its synergistic interaction with Smads to regulate cyclin G2 transcription in ovarian cancer cells. *Oncogene*. ↗

### Editions

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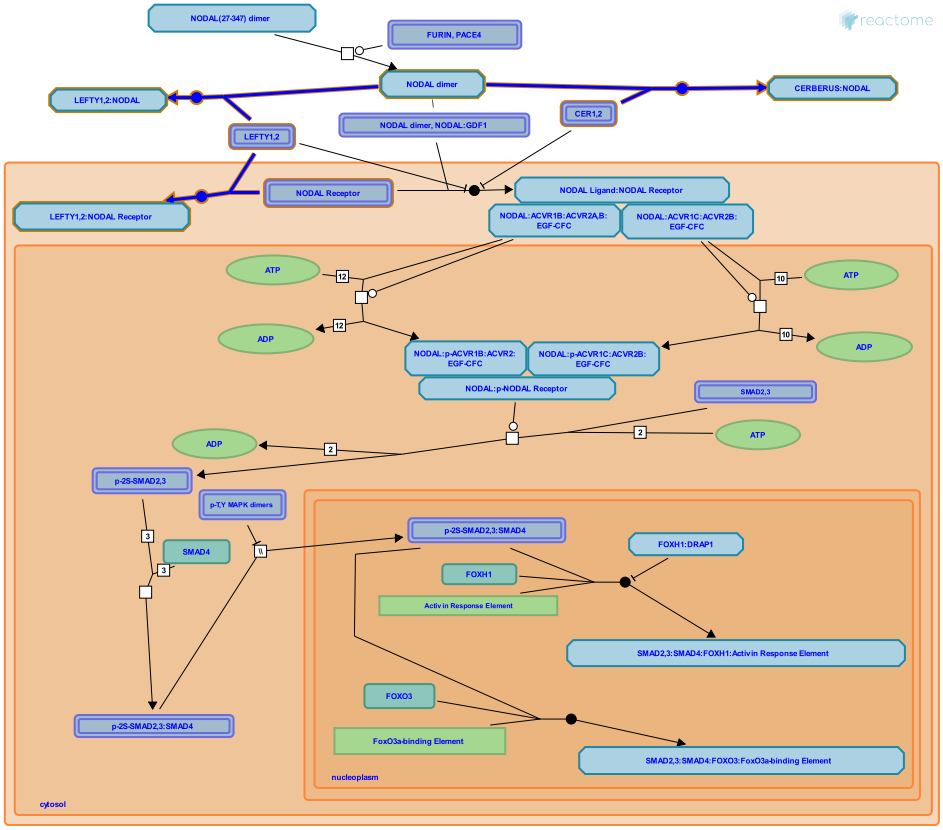
|---|---|---|
| 2011-08-25 | Reviewed | Peng, C. |
| 2011-08-26 | Authored, Edited | May, B. |

## Regulation of signaling by NODAL ↗

**Location:** Signaling by NODAL

**Stable identifier:** R-HSA-1433617

**Compartments:** plasma membrane, extracellular region




Mature NODAL can form heterodimers with LEFTY1, LEFTY2, or CERBERUS. The heterodimers do not activate the NODAL receptor. LEFTY1 and LEFTY2 also bind CRIPTO and CRYPTIC coreceptors and prevent them from interacting with other components of the NODAL receptor. By these mechanisms LEFTY1, LEFTY2, and CERBERUS negatively regulate NODAL signaling (reviewed in Shen 2007, Schier 2009).

## Literature references


Shen, MM. (2007). Nodal signaling: developmental roles and regulation. *Development, 134*, 1023-34. ↗

Schier, AF. (2009). Nodal morphogens. *Cold Spring Harb Perspect Biol, 1*, a003459. ↗


## Editions

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|---|---|---|
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