Smad signalling network

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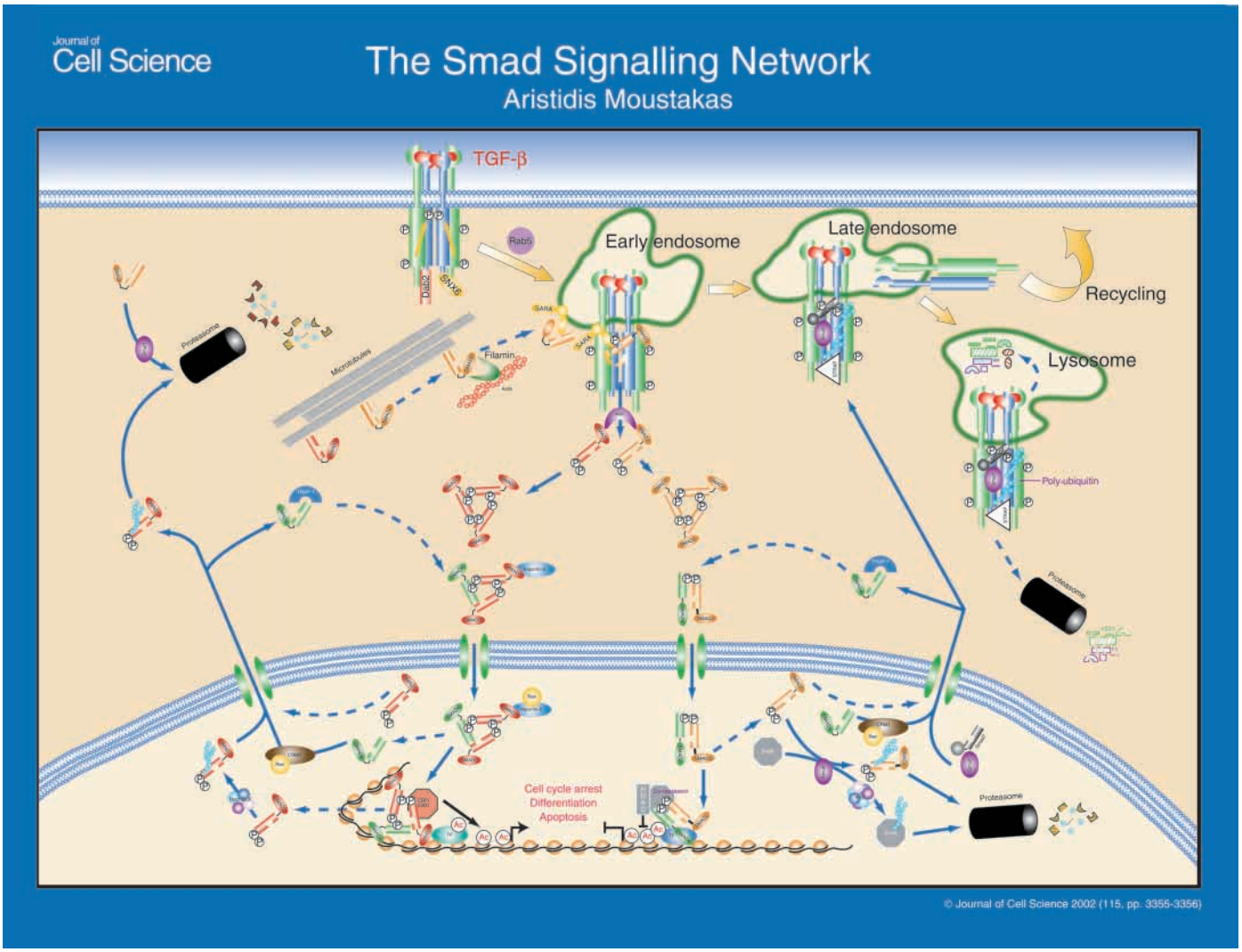
Dimeric ligands of the transforming growth factor β (TGF-β) superfamily bind with high affinity to type II and type I receptor serine/threonine kinases on the cell surface and induce hetero-tetrameric receptor complexes. In such complexes, constitutively auto-phosphorylated type II receptors (green) trans-phosphorylate (arrows) the type I receptor (blue) GS domain. Activated receptors recruit adaptors such as disabled-2 (Dab-2) and sortin nexin 6 (SNX6) that positively affect signal transduction. Small GTPases such as Rab5 catalyse movement of activated receptor complexes to early endosomal compartments, where they encounter phospholipid-bound carriers such as SARA that assist in presentation of the major signalling effectors, the Smads, to the type I receptor kinase. Smads can be anchored in the cytoplasm onto microtubules and actin-binding proteins such as filamin, but the role of the cytoskeleton in Smad signalling is presently unclear (broken arrows).

Type I receptors phosphorylate a subclass of Smads called receptor-activated Smads (R-Smads), such as Smad2 and Smad3, in the TGF-β pathway. R-Smads bind to the phosphorylated GS domain and the L45 loop of the receptor via their phospho-serine-binding C-terminal domain, called the Mad-homology 2 (MH2) domain (rectangle). In addition, Smads consist of the N-terminal MH1 domain (oval) and the divergent linker (thin line). Eventually, the type I receptor phosphorylates the R-Smads (curved arrow) at both serine residues of their conserved C-terminal SXS motifs (X is any amino acid). This changes the R-Smad conformation and leads to its rapid dissociation from the receptor and SARA. The C-terminal phosphoserines are recognised by the MH2 domain of another Smad leading to homo-oligomerisation of R-Smads or hetero-oligomerisation with the unique (in mammals) common-partner-Smad (Co-Smad), Smad4. Smad4 is anchored to the cytoplasm by scaffolding proteins such as TRAP-1 and is recruited to the



(See poster insert)

activated R-Smads by unknown mechanisms (broken arrows). However, TRAP-1 can assist positively in R-Smad/Co-Smad oligomerisation. The Smad4 MH2 domain binds with high affinity to the R-Smad phosphorylated tails and thus forms hetero-oligomeric Smads. The stoichiometry of such oligomers remains ambiguous as Smad4 is reported to form heterotrimers with two Smad3 monomers and heterodimers with one Smad2 monomer. Whether this is a first point of functional divergence between the two R-Smads of the TGF-β pathway or a difference based on experimental conditions remains to be solved. Future refinement of the oligomerisation model under in vivo conditions is awaited.

Phosphorylated Smad3 associates with importin-β1 and is imported to the nucleus. The Ran GTPase catalyses the transport and release of the Smad3 complex in the nucleoplasm. In contrast, phosphorylated Smad2 fails to bind to importins owing to a structural modification of its MH1 domain, and is autonomously imported to the nucleus. In the ground state, Smad4 enters the nucleus constitutively (not shown) and is immediately exported back to the cytoplasm by the exportin CRM1. After TGF-β stimulation, Smad4 enters the nucleus in complex with R-Smads via a mechanism that probably depends on the nuclear import path of the R-Smad. Whether the nuclear R-Smads can also be exported back to the cytoplasm remains unclear (broken arrows).

When in the nucleus, the R-Smad/Co-Smad complexes regulate gene expression. Both Smad3 and Smad4 bind to DNA sequences termed the Smad-binding elements (SBE) via conserved structural elements in their MH1 domains. In contrast, Smad2 fails to bind to SBEs owing to the structural modification of its MH1 domain but it participates in DNA-bound complexes via its interaction with Smad4. In addition, both R-Smads and the Co-Smad can interact with many general and tissue-specific transcription factors (TF) via their MH1 or MH2 domains. For simplicity, the poster indicates interaction of a DNA-bound transcription factor with the MH1 domain of Smad3. Non-DNA-binding transcription factors also associate with nuclear Smads and their MH2 domains recruit co-activators (such as p300) that lead to acetylation of nucleosomal histones and/or associated transcription factors, events crucial for transcriptional induction. Alternatively, the MH2 domains of nuclear Smads recruit co-repressors that associate with histone deacetylases (HDACs), thus leading to transcriptional repression of target genes. This mode of action of the nuclear Smads explains many physiological effects of the TGF-β superfamily pathways, which include regulation of cell growth, differentiation and apoptosis. Both Smad2 and Smad3 nuclear complexes are known to participate in positive and negative regulation of target genes and the dichotomy shown in the poster serves only artwork purposes.

Nuclear Smads also participate in ubiquitination reactions that lead to downregulation of the pathway itself or to degradation of other target proteins such as transcription factors. Such ubiquitination events may primarily involve R-Smads that are not in complex with the Co-Smad. Whether this pool of R-Smads is produced after dissociation of the nuclear Smad hetero-complexes (broken arrows) or via direct import of homomeric R-Smads (not shown), is not presently understood. Nuclear phosphorylated Smad3 becomes ubiquitinated by the Roc1/SCF E3 ligase and is exported to the cytoplasm for proteasomal degradation. Cytoplasmic R-Smads in the ground cell state can be attacked by a Smad-specific E3 ligase family, the Smurfs, which also lead to proteasomal degradation of R-Smads, and thus keep the available R-Smad pools low. Smurfs also ubiquitinate phosphorylated nuclear R-Smads (for simplicity, only Smad2 is shown) that are degraded in the nucleus. Alternatively, nuclear R-Smad/Smurf complexes attack transcriptional repressors such as SnoN, and thus downregulate the repressor. Such events are important to remove inhibitory activities from target genes that need to be transcriptionally induced by Smad complexes.

A third class of Smads, the inhibitory Smads (I-Smads), such as Smad7, reside in the nucleus and also associate with Smurfs. The Smad7-Smurf complex can be exported to the cytoplasm after TGF-β stimulation (unknown mechanism) and targets the TGF-β receptors, possibly at late endosomal compartments. Smad7 essentially lacks an MH1 domain (grey sphere) but has a conserved MH2 domain. By binding to the type I receptors Smad7 inhibits recruitment and phosphorylation of R-Smads (not shown). In addition, via Smurfs, Smad7 mediates receptor ubiquitination. Another adaptor protein, STRAP-1, also binds to both type I receptors and Smad7, and enhances the inhibitory activity of Smad7. Poly-ubiquitination of receptors leads to lysosomal targeting of the complex; subsequently, proteolysis of receptors and possibly the ligand are thought to occur by lysosomal and proteasomal activities. Recycling of non-activated receptors to the plasma membrane is also a documented event that still lacks detailed molecular dissection. Thus, the signalling pathway is shut down and starts preparing for new cycles of signal transduction.

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