1. Phylogeny  
   The RET proto‐oncogene encodes a receptor tyrosine‐protein kinase that is highly conserved across metazoans. Comparative sequence analyses have identified orthologs of RET in a wide range of vertebrate species—including mammals, birds, amphibians, and fish—as well as more divergent homologs in invertebrates such as Drosophila. In vertebrates, RET plays a crucial role in neurodevelopment and organogenesis, and the conservation of key motifs in its extracellular cadherin‐like domains (CLDs) and intracellular kinase domain underscores its fundamental role in signal transduction (Flores2015retdependenttranscriptional pages 81-83). Phylogenetic studies classify RET within the receptor tyrosine kinase (RTK) superfamily, where it shares common ancestral origins with other RTKs such as the epidermal growth factor receptor (EGFR) family and fibroblast growth factor receptors (FGFRs). Its evolutionary lineage is marked by the emergence of a sophisticated extracellular region that contains calcium‐dependent CLDs followed by a cysteine‐rich domain (CRD), a configuration that is unique within the RTK family and critical for ligand and coreceptor recognition (Jhiang2000theretprotooncogene pages 4-5). Additionally, alternative splicing of the RET gene gives rise to multiple isoforms (e.g., RET9, RET43, and RET51), a diversification that appears to be conserved among vertebrates and suggests an evolutionary pressure to fine‐tune RET signaling for tissue‐specific functions (Jhiang2000theretprotooncogene pages 5-6, Flores2015retdependenttranscriptional pages 81-83).
2. Reaction Catalyzed  
   The catalytic activity of RET is defined by its ability to perform ATP‐dependent phosphorylation of tyrosine residues. In its active state, RET transfers the γ‐phosphate group from ATP to the hydroxyl group of specific tyrosine residues on its own intracellular domain (autophosphorylation) as well as on downstream substrate proteins. This reaction can be formally written as:  
     ATP + [protein]‐tyrosine → ADP + [protein]‐phosphotyrosine + H⁺  
   This phosphotransfer reaction is the hallmark of receptor tyrosine kinases and is critical for creating phosphotyrosine docking sites that recruit SH2‐ and PTB‐domain–containing adaptor proteins, thereby initiating a cascade of downstream signaling events (Prescott2015theretoncogene pages 1-2, Iavarone2006activationofthe pages 1-2, Schlumberger2021theimportanceof pages 1-5).
3. Cofactor Requirements  
   The kinase activity of RET depends on the presence of divalent metal ions, with Mg²⁺ being the primary cofactor required for catalysis. Mg²⁺ ions coordinate with the phosphate groups of ATP and stabilize its binding in the active site, thereby facilitating the proper orientation of the nucleotide for efficient phosphoryl transfer. Without Mg²⁺, ATP binding and subsequent phosphorylation become energetically unfavorable, leading to a loss of catalytic activity (Schlumberger2021theimportanceof pages 1-5, Mahato2020retreceptortyrosine pages 1-4).
4. Substrate Specificity  
   RET exhibits substrate specificity through its intrinsic preference for phosphorylating tyrosine residues. In its autophosphorylation process, RET modifies multiple tyrosine residues—including, for example, Y905, Y1015, Y1062, and Y1096—in its intracellular domain. These phosphorylated tyrosines serve as high‐affinity docking sites for downstream adaptor proteins that contain SH2 or PTB domains, thereby propagating key signaling pathways. Although a strict linear consensus substrate motif for RET has not been universally defined as it has been for some serine/threonine kinases, the overall substrate recognition is governed by the spatial arrangement of the kinase domain and the accessibility of specific tyrosine residues on target proteins. The recruitment of adaptor proteins is largely determined by the surrounding amino acid context of these phosphorylated sites rather than a fixed primary sequence motif, resulting in the activation of diverse downstream pathways such as the MAPK/ERK and PI3K/AKT cascades (Jhiang2000theretprotooncogene pages 5-6, Prescott2015theretoncogene pages 1-2, Flores2015retdependenttranscriptional pages 20-24).
5. Structure  
   The overall structure of RET is defined by a modular organization that is integral to its function. Extracellularly, RET comprises four cadherin-like domains (CLD1–CLD4) arranged in tandem, followed by a cysteine-rich domain (CRD) that provides the structural framework for ligand and coreceptor binding. The calcium-binding site located between the CLD2 and CLD3 domains is critical for proper folding, processing, and receptor export, ensuring that RET achieves a functional conformation at the cell surface (Plazamenacho2018structureandfunction pages 1-2). This extracellular configuration, which distinguishes RET from many other RTKs, allows it to form a ternary complex with glial cell line‐derived neurotrophic factor (GDNF) family ligands and their corresponding GPI‐anchored coreceptors (e.g., GFRA1–4), a prerequisite for receptor dimerization and activation.  
   Following the extracellular region, a single hydrophobic transmembrane segment anchors RET in the plasma membrane. Intracellularly, RET exhibits a juxtamembrane region that precedes a highly conserved tyrosine kinase domain. This kinase domain adopts the typical bilobal structure seen in protein kinases, with a small N-terminal lobe predominantly composed of β-sheets that contains the glycine-rich loop (P-loop) responsible for ATP binding, and a larger C-terminal lobe that is enriched in α-helices and harbors the activation loop. The kinase active site is defined by key catalytic residues, including a conserved lysine (e.g., K758 in human RET) located in the P-loop that interacts with the phosphates of ATP and a glutamate located in the αC helix (E775) which forms a critical salt bridge with the lysine. Furthermore, motifs such as the DFG sequence in the activation segment coordinate Mg²⁺ ions required for catalysis, while the HRD motif in the catalytic loop provides the nucleophilic environment essential for phosphoryl transfer (Plazamenacho2018structureandfunction pages 2-3, Iavarone2006activationofthe pages 1-2). In addition, alternative splicing of the RET gene results in isoforms (RET9, RET43, RET51) that differ primarily in the length and sequence composition of the C-terminal tail, a variation that has implications for receptor trafficking, signaling specificity, and subcellular localization (Jhiang2000theretprotooncogene pages 5-6, Prescott2015theretoncogene pages 1-2).
6. Regulation  
   The regulation of RET kinase activity is multifaceted, with ligand binding, alternative splicing, and post‐translational modifications all playing critical roles. RET activation is initiated when GDNF family ligands—such as GDNF, neurturin (NRTN), artemin (ARTN), persephin (PSPN), and GDF15—bind to their corresponding glycosylphosphatidylinositol-anchored coreceptors (GFRA1–4 or GFRAL), forming a tripartite complex that triggers receptor dimerization. This dimerization relieves autoinhibitory constraints within the receptor, allowing trans‐autophosphorylation of key tyrosine residues in the intracellular domain (Drilon2018targetingretdrivencancers pages 1-3, Prescott2015theretoncogene pages 1-2, Schlumberger2021theimportanceof pages 1-5).  
   Autophosphorylation proceeds in a sequential manner. An initial wave of phosphorylation enhances the intrinsic catalytic activity of the kinase domain, while a subsequent wave results in the phosphorylation of tyrosine residues that serve as docking sites for downstream signaling molecules. Post‐translational modifications are further involved in regulating RET function; for instance, glycosylation at multiple sites within the extracellular region is essential for proper folding, receptor maturation, and cell surface expression, with mature RET exhibiting a higher molecular weight relative to its immature form (Prescott2015theretoncogene pages 1-2). Ubiquitination pathways also participate in receptor turnover by marking RET for endocytosis and proteasomal degradation after activation (Santarpia2013inhibitionofret pages 2-2). In pathological contexts, activating mutations such as the M918T substitution (commonly associated with multiple endocrine neoplasia type 2B) or cysteine mutations present in MEN2A can bypass normal regulatory mechanisms, leading to constitutive kinase activity and aberrant downstream signaling even in the absence of ligand (Iavarone2006activationofthe pages 1-2, Jhiang2000theretprotooncogene pages 5-6, Sahakian2023molecularbasisand pages 2-4). Moreover, intramolecular allosteric regulation and conformational transitions—particularly involving the activation loop—further modulate RET activity by controlling access to the ATP-binding site and the alignment of catalytic residues (Drilon2018targetingretdrivencancers pages 17-19).
7. Function  
   RET serves as a central mediator of cellular signaling pathways that regulate proliferation, differentiation, migration, and survival. Activation of RET by its cognate ligands in conjunction with coreceptors initiates a cascade of phosphorylation events that engage multiple downstream signaling pathways, most notably the MAPK/ERK and PI3K/AKT routes. These pathways are instrumental in modulating gene expression and cytoskeletal dynamics, which in turn influence critical developmental processes such as neuronal migration, axon guidance, and the formation of the enteric nervous system, as well as kidney morphogenesis (Sahakian2023molecularbasisand pages 2-4, Frilling2003prophylacticthyroidectomyin pages 2-4). RET is predominantly expressed in cells derived from the neural crest and in tissues such as the central and peripheral nervous systems, renal tissues, and certain endocrine cells. Through its ability to recruit adaptor and effector proteins upon autophosphorylation, RET orchestrates a network of downstream signaling modules that can affect cell cycle progression, survival, and differentiation (Drilon2018targetingretdrivencancers pages 1-3, Jhiang2000theretprotooncogene pages 5-6). In the context of human disease, dysregulated RET signaling is strongly implicated in oncogenesis. Activating mutations and gene rearrangements involving RET are a well‐characterized etiology for medullary thyroid carcinoma (MTC) and are also observed in other malignancies, including papillary thyroid carcinoma and certain subtypes of non‐small cell lung cancer (Prescott2015theretoncogene pages 1-2, Schlumberger2021theimportanceof pages 1-5). The versatility of RET signaling also extends to non-oncogenic contexts such as neuronal survival and differentiation, highlighting its dual roles in both development and disease (Mahato2020retreceptortyrosine pages 1-4).
8. Other Comments  
   Multiple small-molecule inhibitors have been developed to target RET’s kinase activity, driven by the need to modulate aberrant signaling in RET-driven cancers. Clinically approved multikinase inhibitors—such as vandetanib, cabozantinib, and lenvatinib—exhibit RET inhibitory activity alongside effects on other kinases and have been evaluated for use in medullary thyroid carcinoma and RET fusion–positive lung cancers (Drilon2018targetingretdrivencancers pages 7-9, Santarpia2013inhibitionofret pages 7-8). In addition, newer selective RET inhibitors are under active investigation to overcome resistance mechanisms associated with specific gatekeeper mutations (e.g., V804M), which can limit the efficacy of less selective agents (Santarpia2013inhibitionofret pages 9-11). RET is also notable for its involvement in developmental disorders; for example, loss-of-function mutations have been linked to Hirschsprung disease, while gain-of-function mutations are implicated in multiple endocrine neoplasia types 2A and 2B (Iavarone2006activationofthe pages 1-2, Jhiang2000theretprotooncogene pages 5-6, Sahakian2023molecularbasisand pages 2-4). Furthermore, alternative splicing of the RET transcript produces isoforms with differential signaling capacities and distinct subcellular localization patterns, aspects that continue to influence therapeutic strategies and the design of targeted inhibitors (Jhiang2000theretprotooncogene pages 5-6, Prescott2015theretoncogene pages 1-2).
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