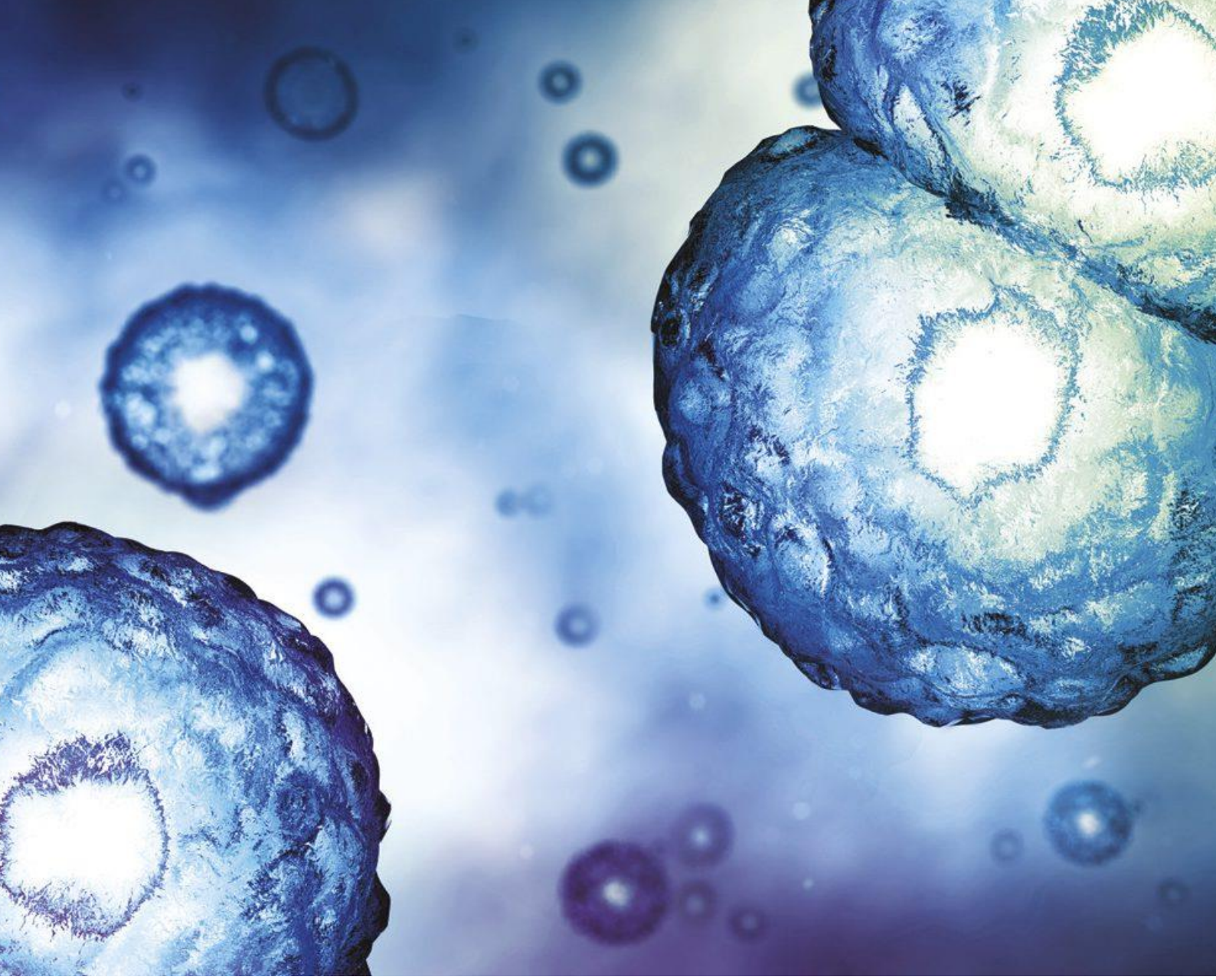

The insulin and IGF-1 receptors

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A bit of history The birth of the receptor concept dates back to the early work of John Newport Langley (1852-1925), a Cambridge physiologist, who postulated in 1905 that a "receptive substance" on the surface of skeletal muscle mediated the action of nicotine (1). At about the same time, Paul Ehrlich (1854-1915), a German immunologist who was the founding father of chemotherapy, came up with a "side chain theory" of cell receptors to explain the selectivity of immune reactions, winning him the Nobel Prize in Physiology or Medicine in 1908. His famous adage "Corpora non agunt nisi fixata" ("Substances do not act unless they are bound") is an elegant and concise early statement of the receptor theory (2).

The receptor concept was put on more solid ground with the seminal 1948 paper of Raymond P. Ahlquist (1914-1983), an American pharmacologist of Swedish descent at the Medical College of Georgia, who proposed that the excitatory and inhibitory effects of adrenotropic agents were mediated by two separate receptors which he termed a and b (3). The receptors would however remain hypothetical entities until the late 60's, when direct methods to study their biochemistry were developed.

The concept that insulin exerts its effects by acting at the membrane of target cells was proposed over 60 years ago by Rachmiel Levine (1910-1991), considered by many as one of the founding fathers of modern diabetology, then working at Walter Reese Hospital in Chicago. He postulated that "insulin acts upon the cell membrane of certain tissues (skeletal muscle, etc.) in such a manner that the transfer of hexoses (and perhaps other substances) from the extracellular fluid into the cell is facilitated" (4). The direct demonstration of cell membrane receptors for polypeptide hormones took another twenty years. In 1969, two groups independently established methods for the radioiodination of peptide hormones with preserved bioactivity: Robert J. Lefkowitz, Jesse Roth and Ira Pastan at the National Institutes of Health in Bethesda (working on ACTH) (5) and Theodore L. Goodfriend´s group at the University of Wisconsin in Madison (working on angiotensin) (6). In 1970 and 1971, three groups independently published the first reports on ^{125}I-insulin binding to liver plasma membranes: P.D.R. House and M.J. Weidemann in Canberra (7), Pierre Freychet, Jesse Roth and David M. Neville Jr at the NIH (8), and

Pedro Cuatrecasas, Bernard Desbuquois and Folger Krug at Johns Hopkins University (9). Shortly afterwards in Denmark, Steen Gammeltoft and Jørgen Gliemann established independently radioligand binding assays for studying insulin receptors on isolated rat fat cells (10). Radioligand binding assays also established the existence of a separate receptor for somatomedins (IGFs) (11,12).

We will not give here a detailed account of the following four decades of productive research on the biochemistry of the insulin and IGF-I receptors, culminating in the cloning of the cDNAs of the two receptors in 1985 (13-15) as well as the elucidation of the crystal structure of their kinase domain (16) and that of the extracellular domain of the insulin receptor (17) and of the N-terminal domain of the IGF-I receptor (18) (see below). For further reading, see ref. 19.

Structure of the insulin and IGF-I receptors

The primary sequence of the insulin receptor was determined by cDNA cloning in 1985 simultaneously by two independent groups, that of Axel Ullrich at Genentech (13) and that of William J. Rutter at UCSF (14). Ullrich's group solved the cDNA sequence of the IGF-I receptor the following year (15).

The gene for a mammalian related receptor (insulin receptor-related receptor or IRR) was identified in 1989 (20). No ligand for this receptor has been identified. The mouse IRR knockout has no phenotype, but the triple IR/IGF-IR/IRR knockout results in gonadal dysgenesis and male-to-female somatic sex reversal, providing the first glimpse of a function for this orphan receptor (21).

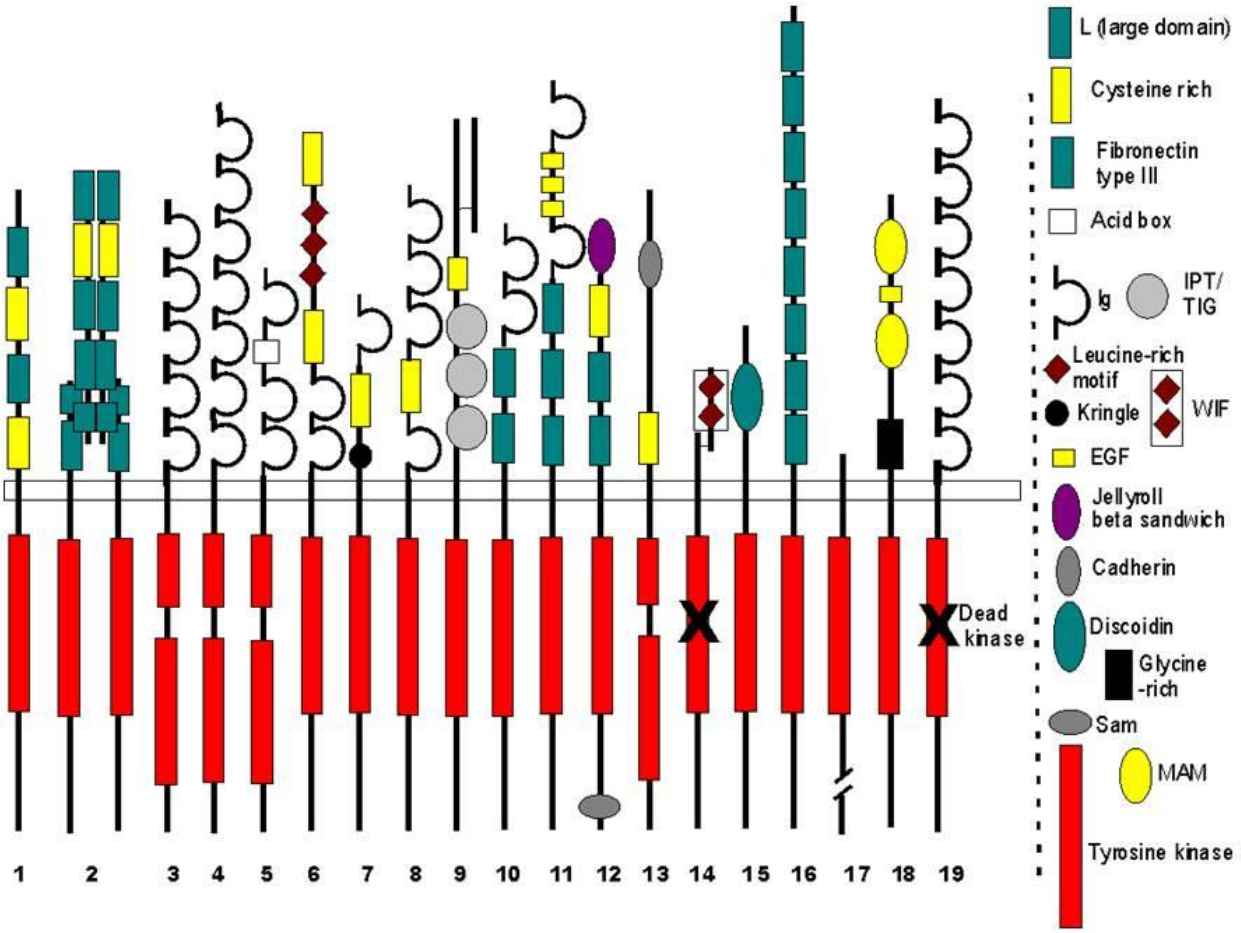
The insulin receptor, the IGF-I receptors and the IRR are members of the family of receptor tyrosine kinases (RTKs) (16). This family comprises in humans 59 members, grouped in 19 subfamilies depending on the architecture of the extracellular domains (Fig. 1).

Figure 1: The receptor tyrosine kinase family. The protein modules that comprise the extracellular domains are described on the right side. From ref. 27, adapted from ref. 16 with corrections and updates from multiple references and databases such as Pfam.

The 19 subfamilies shown are the following:

1.ErbB (HER, EGF receptor), ErbB-2 (HER-2/Neu), ErbB-3 (HER-3), ErbB-4 (HER-4, Tyro-2)(ErbB-3 is a dead kinase)	11.Tie-1, Tek (Tie-2, angiopoietin receptor)
2.INSR, IGF-IR, IRRR	12.EphA1-8, B1-6
3.PDGFRa, PDGFRb, SCF1R (c-Kit), Flk-2	13.Ret (GDNF receptor)
4.VEGFR-1 (Flt-1), VEGFR-2 (Flk-1, KDR) VEGFR-3 (Flt-4)	14. Ryk
5. FGFR-1- 4	15. DDR-1, DDR-2
6.TRKA (NGF receptor), TRKB (BDNF receptor),TRKC (NT-3 receptor)	16. Ros
7. Ror1, Ror2	17. AATYK
8. MusK	18.ALK, LTK
9.Met (HGF/scatter factor receptor), Ron, Sea	19.PTK-7, KLG, CCK-4
10.AxI, Mer (Eyk), Nyk, Rse (Tyro-3)	

The RTKs shown in bold letters have been implicated in malignancies.


L (large domain)
Cysteine rich
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Leucine-rich motif
Kringle
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EGF
Jellyroll beta sandwich
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Discoidin
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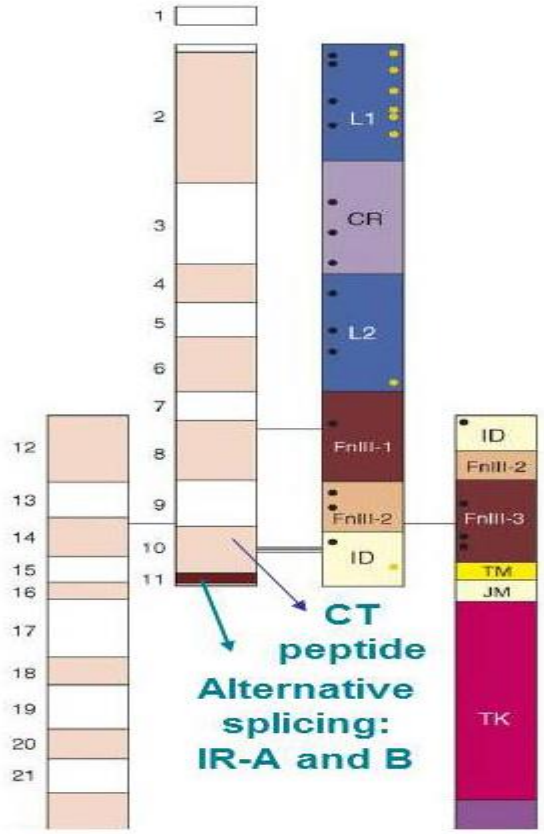
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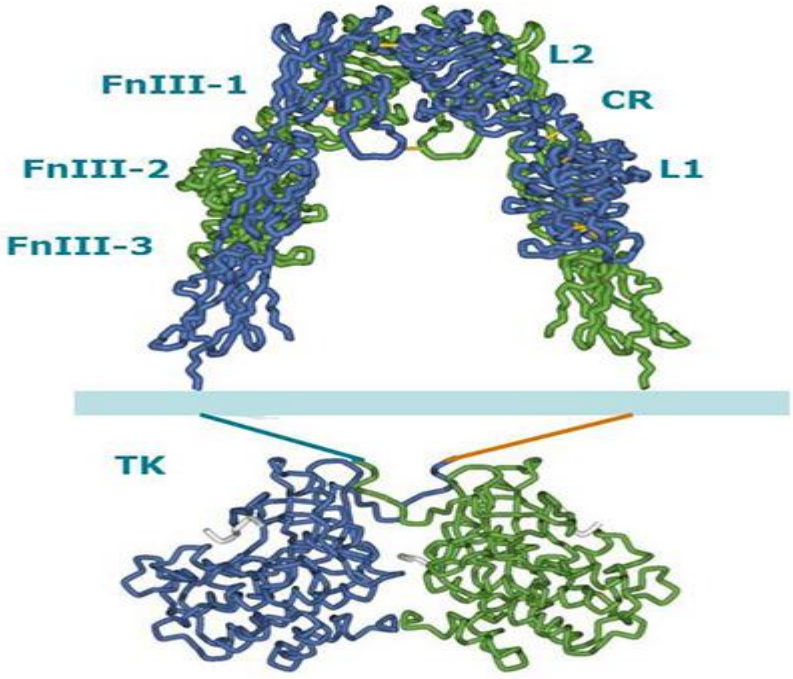
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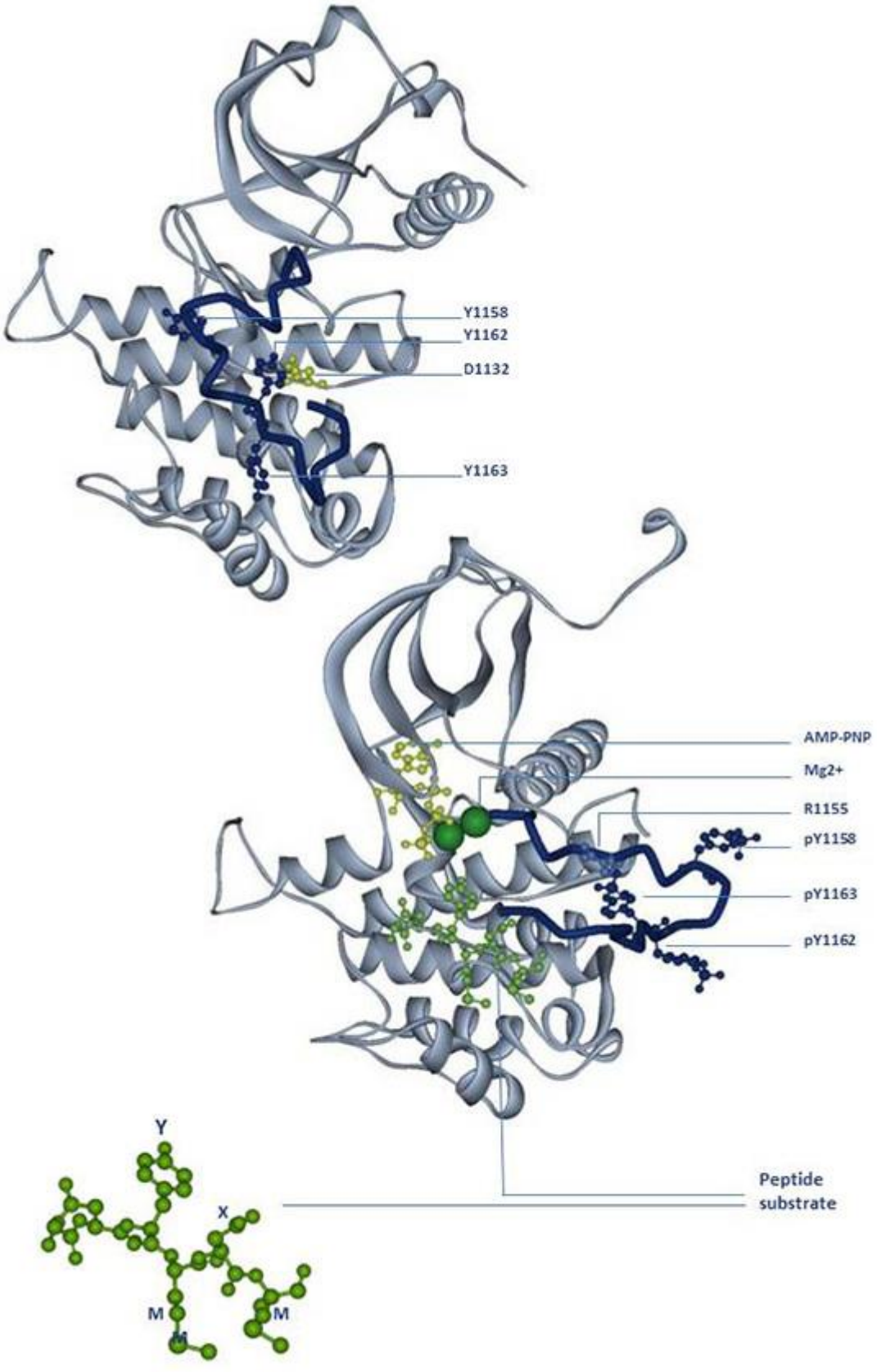
Y1158
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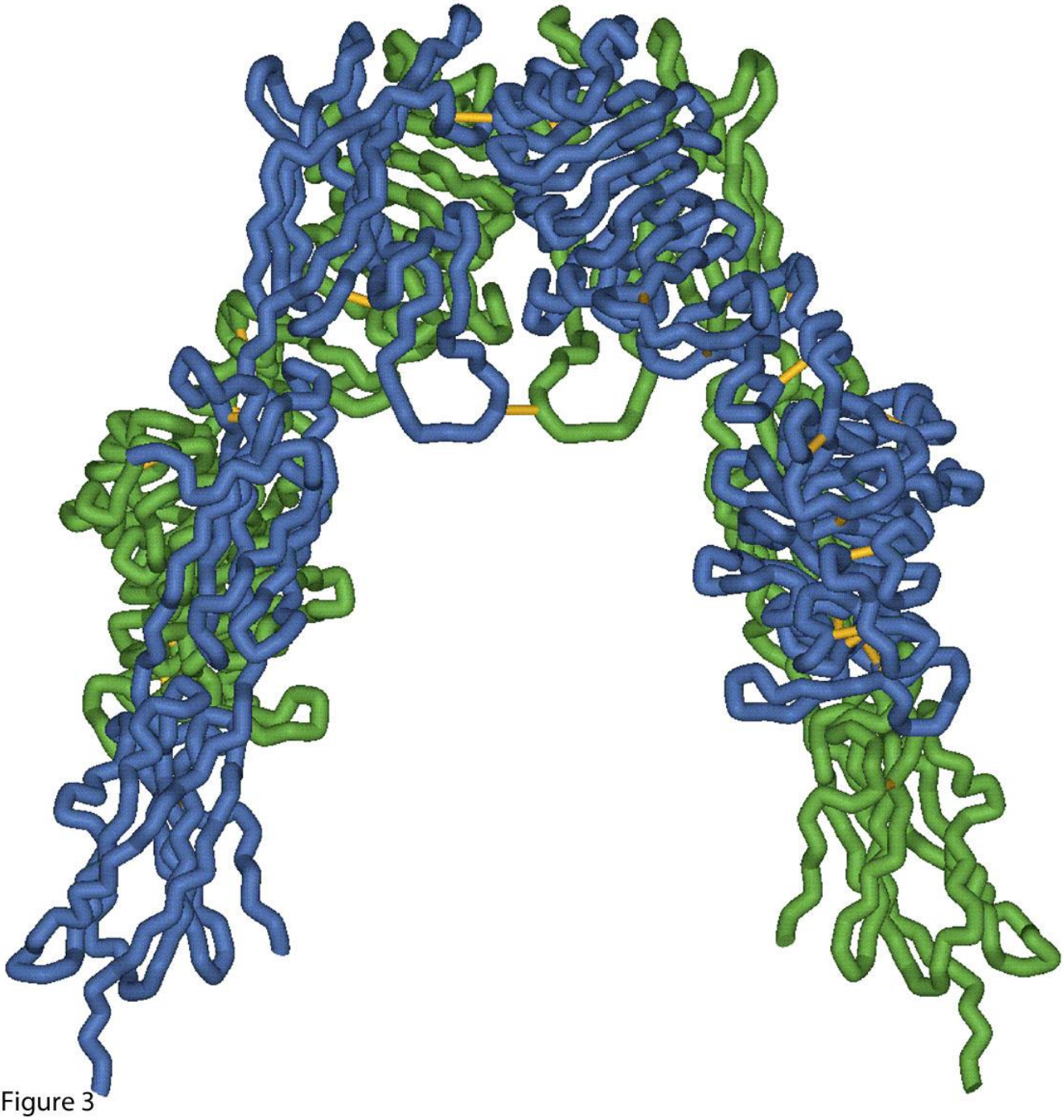
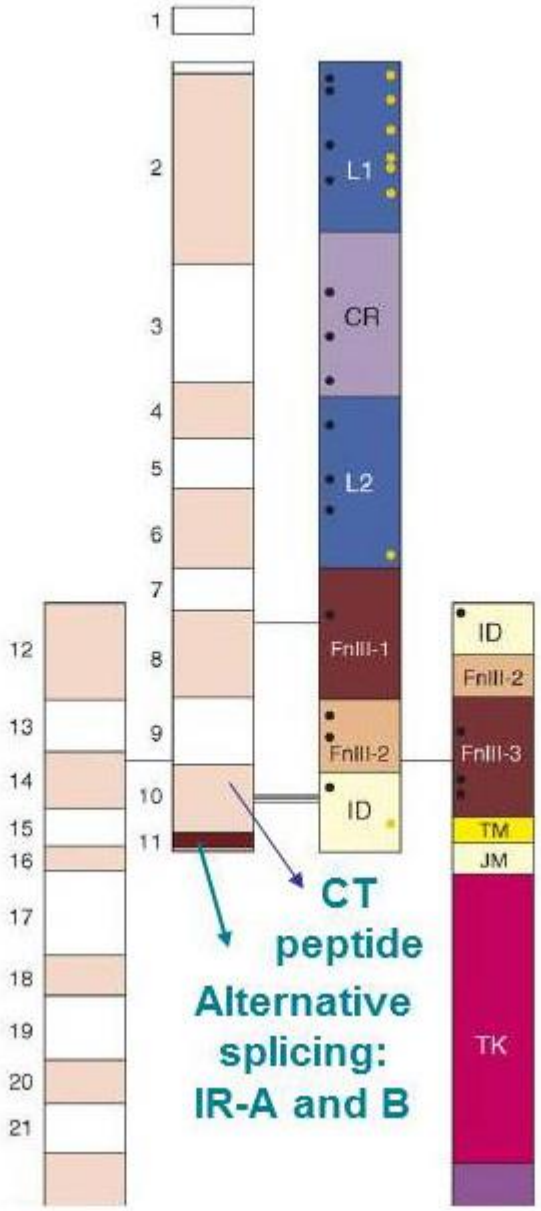


Figure 3

The extracellular domains use a variety of protein modules to compose specific binding sites for the specific ligands of a given family (Fig. 1 and table 1). The intracellular portion contains a tyrosine kinase domain that phosphorylates intracellular protein substrates on tyrosine side chains.

Most of the RTKs are made of single polypeptide chains that cross the cell membrane once, with the exception of the insulin/IGF-I/IRR subgroup that are made of covalent disulfide-bonded dimers made of two extracellular α-subunits and two transmembrane b-subunits (Fig. 2).

Figure 2: Insulin receptor modular structure. Left: schematic structure of the a2b2 insulin receptor dimer. The left half shows the exon-intron boundaries. The right half shows the boundaries of domains defined by secondary structure prediction (ref. 25). L1 and L2: large domains 1 and 2; CR: cysteine-rich domain; FnIII1-3: fibronectin type III domains 1-3; ID: insert domain; CT peptide: C-terminal domain (involved in binding site 1); TM: transmembrane domain; JM: juxtamembrane domain; TK: tyrosine kinase domain. Right: 3-D structure of the extracellular domains (PDB file 2DTG) and the tyrosine kinase domains (PDB file 1IR3) of the insulin receptor. One ab half of the extracellular domain is shown in green, the other one in blue. The RTK domains are shown in corresponding colors.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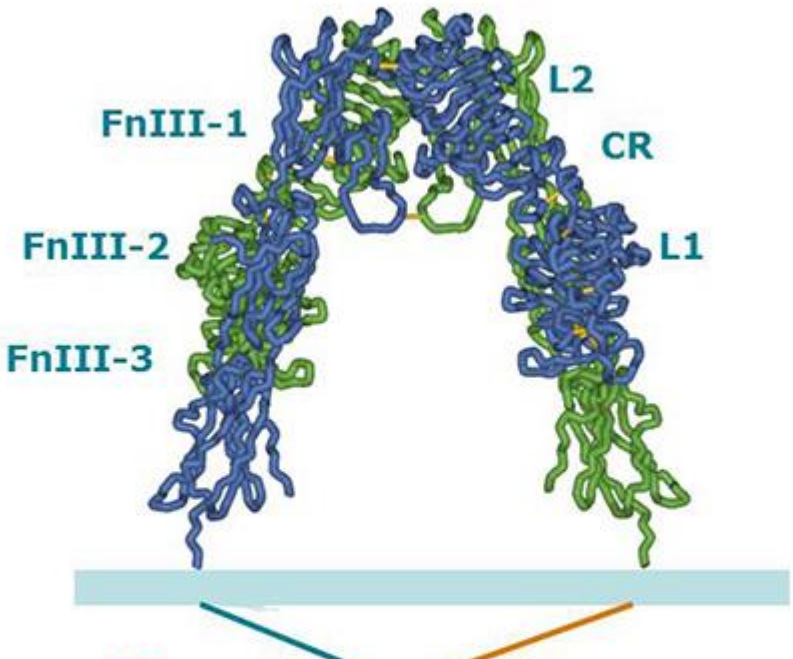
peptide
Alternative
splicing:
IR-A and B


FnIII-1
L2
CR
FnIII-2
L1
FnIII-3

However, all RTKs are dimeric in the active state due to either ligand-induced dimerization or stabilization of preformed noncovalent dimers.

The insulin receptor has a modular structure encoded by a gene with 22 exons and 21 introns (Fig. 2). The short exon 11 is alternatively spliced, resulting in two isoforms (A and B) that differ slightly in affinity for insulin (22). The B isoform binds the IGFs with at least 100 times lower affinity than insulin, while the A isoform has significantly higher affinity than the B isoform for IGF-I and especially IGF-II (23). The IGF-I receptor binds IGF-II with a lower affinity than IGF-I and insulin with a 500-fold lower affinity.

The receptors are synthesized as single chain preproreceptors that are processed, glycosylated, folded and dimerized to yield the mature a_2b_2 receptor.

In cells expressing both insulin and IGF-I receptors, hybrid receptors are formed consisting of one half of each (24). Their physiological role is unknown but their binding properties resemble those of IGF-I receptors.

Comparative sequence analysis of the insulin/IGF-I receptors and the related EGF receptor had led Mona Bajaj in Tom Blundell's group at Birkbeck College (25) to suggest that the N-terminal half of the a-subunits consists of two large homologous globular domains, L1 and L2, separated by a cysteine-rich region predicted to consist of a series of disulfide-linked modules (Fig. 2). These predictions were confirmed by the determination of the crystal structure of the insulin receptor extracellular domain and the N-terminal module of the IGF-I receptor (see below). The C-terminal portion of the extracellular part of the receptor was predicted to consist of three fibronectin type III (FnIII) domains (Fig. 2). The second FnIII domain contains a large insert domain of unknown structure containing the site of cleavage between a- and b-subunits. The intracellular portion of the b-subunit contains the kinase domain flanked by two regulatory regions, a juxtamembrane region involved in docking insulin receptor substrates (IRS) 1-4 and Shc as well as in receptor internalization, and a C-terminal tail.

The crystal structure of the unliganded insulin receptor ectodomain was solved in 2006 at 3.8 Å resolution by Colin Ward's group at the CSIRO in Melbourne, Australia (17). The structure is depicted in Fig. 3.

Figure 3: 3-D structure of the insulin receptor extracellular domain. The X-ray structure of the insulin receptor extracellular domain is shown (PDB file 2DTG). One ab half of the extracellular domain is shown in green, the other one in blue.

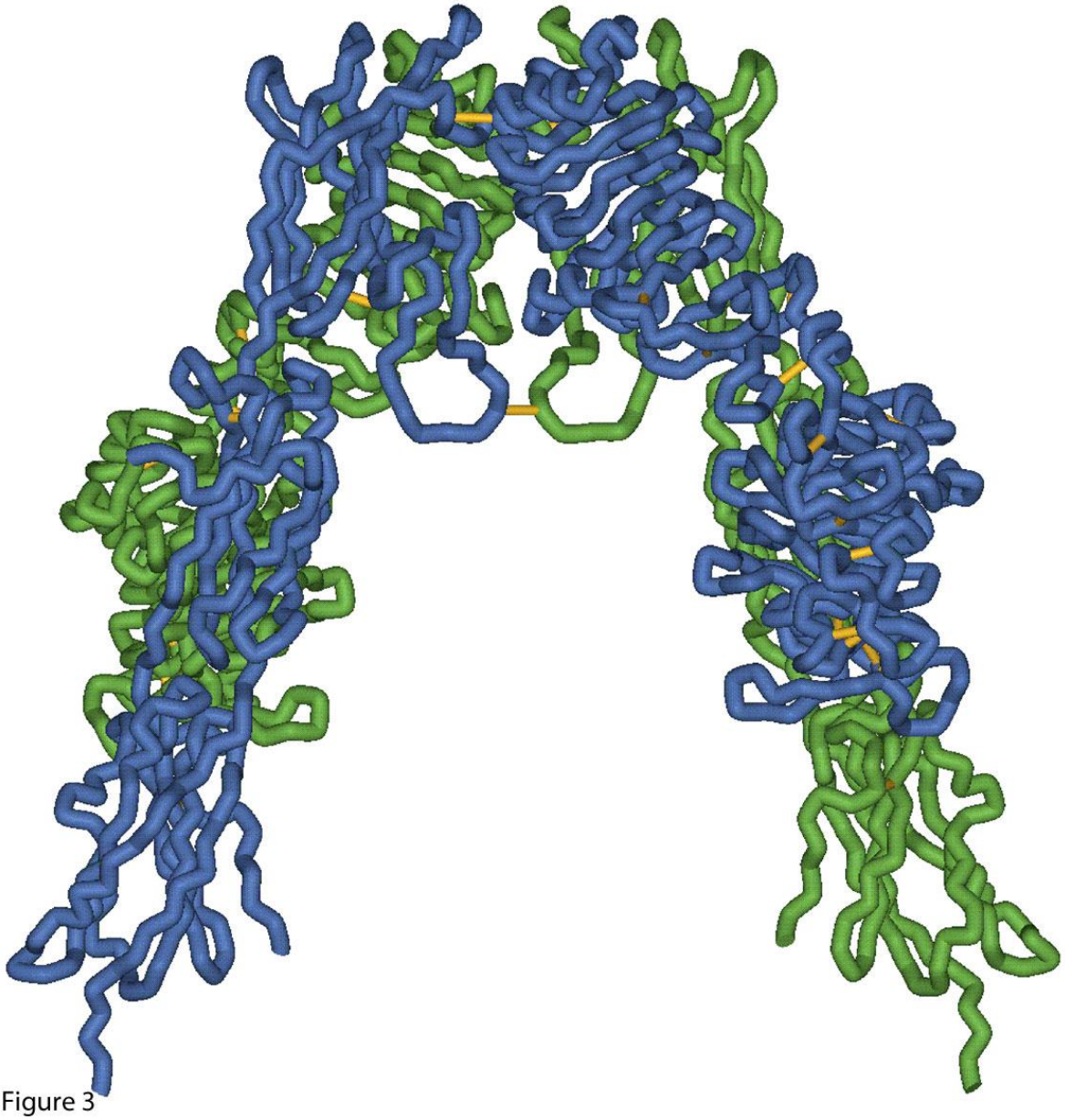


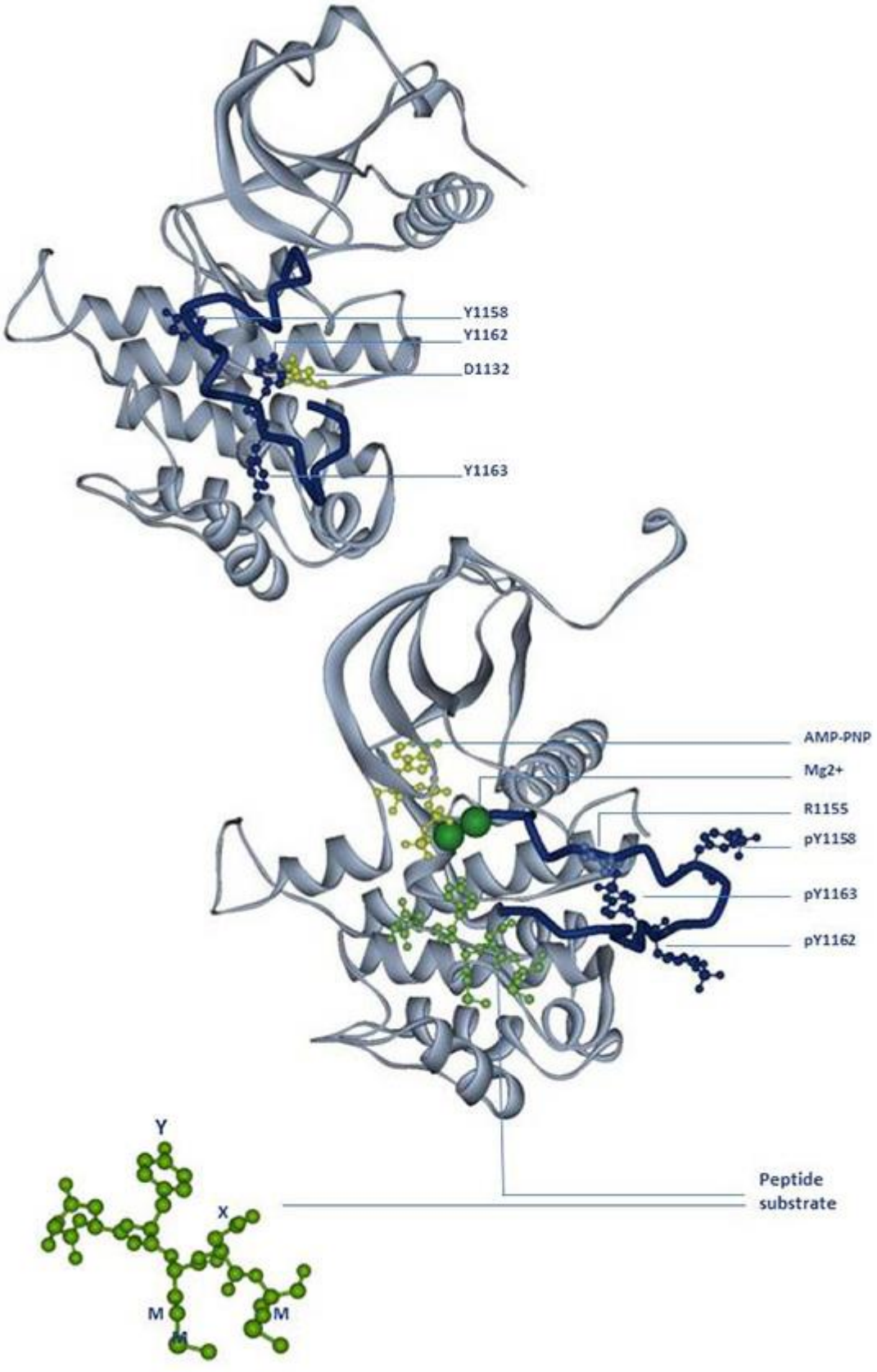
Figure 3

The receptor ectodomain was crystallized in the absence of insulin, but as a complex with four Fabs from monoclonal antibodies 83-7 and 83-14, not shown in Fig. 3, and a fragment of an insulin mimetic peptide, invisible in the structure. Each monomer of the IR adopts a folded-over conformation, making an "inverted V" arrangement relative to the cell membrane; one leg made of the three FnIII domains stems from the membrane (the a-subunit C-terminal portion being superimposed linearly on top of the extracellular part of the b-subunit), while the other leg is formed by the L1-CR-L2 domains. The two monomers are disposed in an antiparallel symmetry consistent with the model proposed by Pierre De Meyts at Novo Nordisk A/S in 1994 (26). A critical element of the binding mechanism is unfortunately invisible due to a disordered structure, the insert domain (ID) in FnIII-2 that contains the a-b cleavage site, the CT domain that is part of binding site 1 (see section on how insulin binds to its receptor) and the triplet of a-b disulfide bonds Cys682, Cys 683 and Cys685.

A crystal structure of the L1-CR-L2 domain of the IGF-I receptor shows a very similar arrangement of this part to that of the insulin receptor (18).

The structures of the tyrosine kinase domain of the insulin and IGF-I receptors, both in the inactive and in the active states have been determined by Stevan Hubbard at New York University (Fig. 4) (for review see ref. 16).

Figure 4: Mechanism of insulin receptor tyrosine kinase (IRTK) activation. The X-ray structures of the inactive (left, PDB file 1IRK)) and activated (right, PDB file 1IR3) IRTK are shown. The activated structure on the right is bound to an ATP analogue, adenylyl imidodiphosphate (AMP-PNP) as well as a peptide substrate YMXM and magnesium. The figure illustrates the autoinhibition mechanism, whereby Tyr (Y) 1162 - one of the three tyrosines (Y1158, Y1162 and Y1163) that are autophosphorylated in the activation loop (in white) in response to insulin - is bound in the active site, hydrogen-bonded to a conserved Asp (D) 1132 residue in the catalytic loop (a). Y1162 in effect competes with protein substrates before autophosphorylation. In the activated state (b), the activation loop is tris-phosphorylated and moves out of the active site. Y1163 becomes hydrogen-bonded to a conserved R1155 residue in the beginning of the activation loop, which stabilizes the repositioned loop. From ref. 28, adapted from ref. 16.


Y1158
Y1162
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Y1163
AMP-PNP
Mg2+
R1155
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pY1163
pY1162
Peptide
substrate
Y
X
M
M
M

They show the bilobed structure typical of protein kinases with the active site as a cleft in the middle, and shed light on the mechanism of kinase activation with an autoinhibitory loop being moved out of the active site when phosphorylated on three tyrosyle residues.

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