Keys to unlocking human dopamine D1 receptor signal transduction

Our body organs, such as the brain, are made of different cells that talk to each other by releasing natural chemicals outside the cells. The free chemicals then attach to specialized proteins named receptors, which are anchored to the membrane surrounding the cytoplasm (interior content) of cells. Attachment of the chemical causes and stabilizes the formation of active receptors, which culminate in the production of a second messenger in the cytoplasm. This process known as signal transduction requires the active receptor to grab other protein types in the cytoplasm such as the G proteins, which in turn recruit an effector that produces a second messenger. The second messenger starts communication inside the cell.

We study the human D1 receptor (D1R), which attaches specifically to dopamine, a brain chemical important for locomotion, learning, memory and pleasure. The D1R poses as a popular target for research because its dysfunction has been implicated in mental disorders including Parkinson's disease, schizophrenia and drug addiction. While much research has focused on creating synthetic chemicals that aim to combat such illnesses, understanding how D1R constituents work to trigger signal transduction is equally important to develop novel therapeutic drugs.

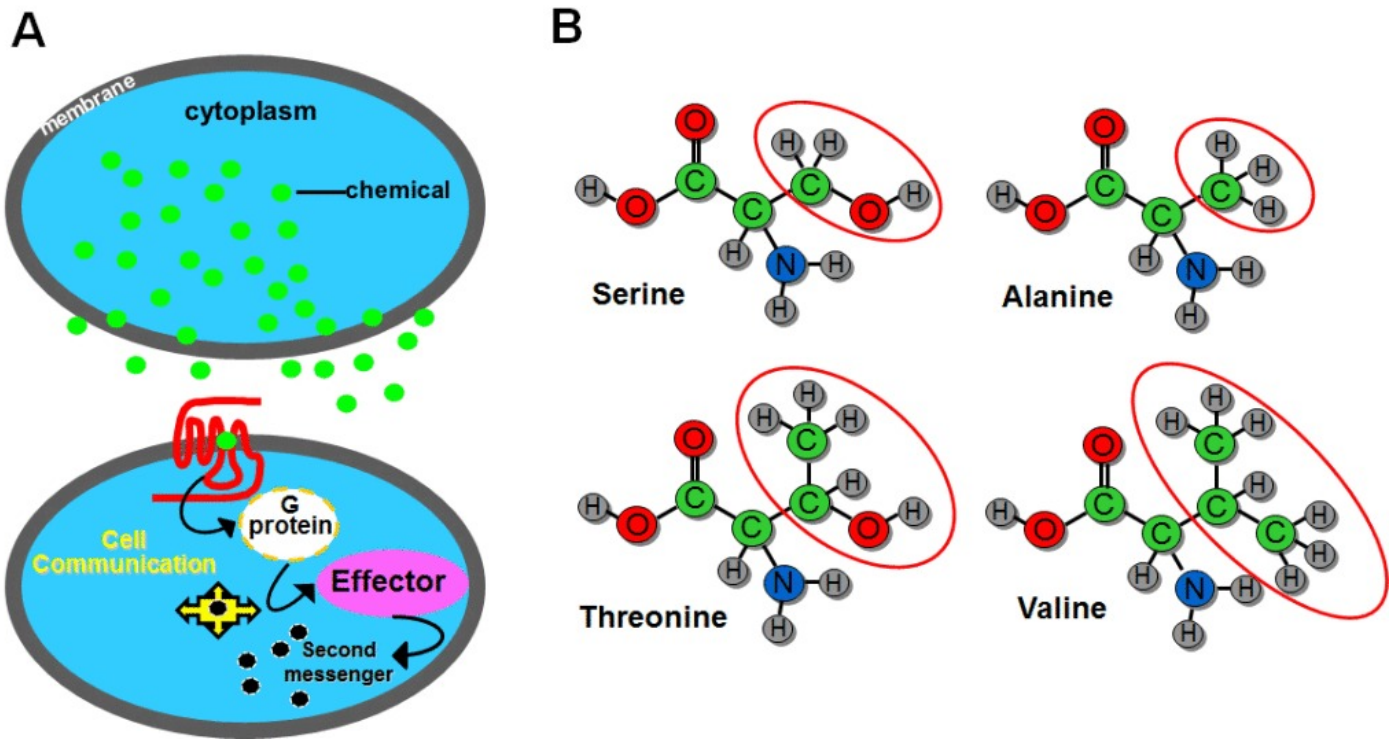

Fig. 1. (A) Schematic representation of signal transduction triggered by the release of a cell chemical (green) and its attachment to a receptor called GPCR (red), which leads to the production of second messengers in cytoplasm with the help of a G protein (white) and an effector (pink). This

cascade of biochemical events controls the communication inside the cell.
(B) The amino acids, serine (Ser), threonine (Thr), alanine (Ala) and valine (Val), are depicted. Differences between their side chains (containing or not hydroxyl groups) are highlighted in red circles. The bonds connecting atoms are represented by black lines.

The human D1R belongs to the large family of **G** **P**rotein-**C**oupled **R**eceptors (a.k.a. **GPCR**s), whose members repeatedly criss-cross the membrane of cells leading to the formation of three segments called the extracellular segment (outer cell segment), the core segment (cell membrane embedded segment), and the intracellular segment (inner cell segment). The extracellular segment is made of a tail and three loops (EL1, EL2 and EL3), while the intracellular segment is composed of a cytoplasmic tail (CT) and as well three loops (IL1, IL2 and IL3). The core segment is formed by seven transmembrane (TM) helices (TM1-TM7) connected by the extracellular and intracellular loops (see Figure 1A). X-rays studies of crystals containing chemicals attached to GPCRs have given a 3D image of the amino acids (building blocks of proteins) and atoms (e.g. oxygen) that make GPCRs and how the side chains of amino acids within GPCRs adopt distinct shapes following the attachment of chemicals. The current view for the activation of GPCRs posits that the activating chemical (called the agonist) attaches to amino acids of the TM helices, which lead to physical changes in helices and intracellular segment. Some of the amino acids and atoms in the cytoplasmic tail and intracellular loops IL2 and IL3 turning on the G protein and formation of the second messenger have been identified. Meanwhile, whether IL1 components contribute to GPCR activation remain unknown. This is an important issue for the future development of drugs specifically targeting D1R in diseases.

In our study, we tackle this issue by investigating two amino acids, serine (Ser) and threonine (Thr) located within and near IL1 of the human D1R located at the amino acid position 65 and 59, respectively. We use a technology called mutagenesis to make two human D1R mutants, in which Ser65 and Thr59 were respectively replaced with alanine (Ala) and valine (Val) to solely remove the hydroxyl chemical group (OH, oxygen + hydrogen) located on their side chain (hydroxyl side chain is highlighted in Figure 1B). We then tested in cells cultured in dishes if the removal of OH from Ser and Thr change how dopamine attaches to and activates these two mutant GPCRs relative to the natural D1R. We found when Thr59 was replaced with Val, the mutant receptor (called D1-T59V) had lower affinity for dopamine in comparison to D1R. The ability of D1R to turn on the G protein and second messenger synthesis in the absence of dopamine was abolished in cells containing D1-T59V. Dopamine was 300-fold less potent for activating D1-T59V as compared to D1R.

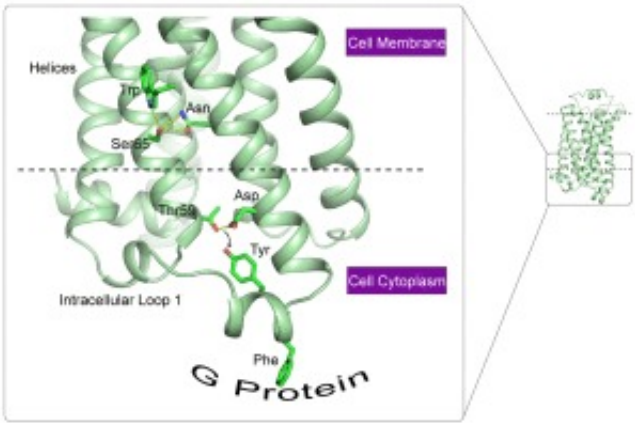

Fig. 2. A computer-generated 3D image showing some of the core and intracellular segments (encapsulated in large box). This represents a zoomed-in view of the lower portion of D1R (shown on the right). The amino acids and atom groups making contact with Thr59 and Ser65 of D1R are depicted using colored sticks following the same coloring scheme as Figure 1B. Curved arrow indicates as D1R becomes active upon attachment of dopamine, Thr59 can switch from the Asp to contact Tyr of IL2 to guide the insertion of Phe into the G protein. TM4 segment has been made transparent to allow better visualization of Thr59 and Ser65. Dashed lines represent the membrane boundary on the cytoplasmic side.

Interestingly, Thr59 of D1R is also found in a similar position in many other GPCRs. As there is no X-rays of D1R in crystals, we rely on available 3D images from other active and inactive forms of GPCRs that are closely related to D1R. This allowed us to develop a theoretical 3D image of the human D1R to uncover how Thr59 interacts with other components of D1R. Our findings suggest that in the inactive form (no dopamine attached) of D1R, the hydroxyl group (OH) located on the side chain of Thr59 interacts with a particular amino acid named aspartate (Figure 2). Once dopamine attaches to D1R, we speculate that the hydroxyl side chain of Thr59 separates from the aspartate to make a contact with another amino acid, tyrosine (Tyr), located in the middle of IL2. This, in turn, helps phenylalanine (Phe), a nearby amino acid of Tyr in IL2, to make a contact with the G protein (Figure 2). We suggest that without the hydroxyl side chain of Thr59 (as in D1-T59V) the Phe of IL2 cannot efficiently make contact with the G protein. This would then diminish the ability of D1R to turn on the G protein and second messenger synthesis.

Capitalizing on Ser65 being also found at a similar position in many other GPCRs, our theoretical 3D image generated for D1R by computer analysis show that Ser65 is situated at the junction of IL1 and TM2 of D1R (Figure 2). Based on this computer model, we propose that in both dopamine-free (inactive) and dopamine-bound (active) D1R states, the hydroxyl side chain of S65 makes contact with the amino acid asparagine (Asn) of TM3 and the amino acid tryptophan (Trp) of TM4 (Figure 2). When Ser65 is changed to Ala (D1-S65A), we observed an increase in dopamine affinity, and most strikingly, a 2-fold higher efficacy of dopamine in producing full stimulation of G

protein and second messenger synthesis compared to D1R. Follow-up studies are underway to identify the mechanisms responsible for the elevated signal transduction of D1-S65A.

In summary, our work has demonstrated that two nearby residues of D1R—Thr59 and Ser65—control the signal transduction of D1R triggered by the attachment of dopamine. Of importance for the field of GPCR research, our study has highlighted a novel role of IL1 and IL1/TM2 junction, notably the conserved GPCR amino acids Thr59 and Ser65, which now needs to be appreciated in our current view of signal transduction by GPCRs. We hope these novel structural determinants can be potentially made use of for developing therapies requiring either increase or decrease signal transduction of D1R such as in Parkinson's disease and schizophrenia, respectively.

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Publication

Functional importance of two conserved residues in intracellular loop 1 and transmembrane region 2 of Family A GPCRs: insights from ligand binding and signal transduction responses of D1 and D5 dopaminergic receptor mutants.
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