COMMENTARY

Cloning the sigma$_2$ receptor: Wandering 40 years to find an identity

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Scientists have endeavored to understand sigma receptors for over 40 y. Although most agree that they are important, there is little agreement on anything else. In their behavioral classification of opioid receptors in 1976, Martin et al. (1) proposed three groups of compounds illustrating three distinct opioid receptor classes (mu, kappa, and sigma) based upon morphine, ketocyclazocine, and SKF10,047, respectively, and noted that the opioid antagonist naltrexone antagonized them all. Since then, the sigma receptor story has undergone many twists and turns. Although the SKF10,047 stereoisomer used in the initial description is not stated, subsequent investigators used (+)SKF10,047 to define sigma receptors, identifying sites that clearly were not opioid. Extensive binding studies associated (+)SKF10,047 with many putative receptors, including phencyclidine (2), but these ligands proved quite promiscuous, labeling a multitude of sites. As more ligands became available, sigma receptors were dissociated into two categories: sigma$_1$ and sigma$_2$ (3). Functional studies strongly suggested that both classes were important. Our understanding of sigma$_1$ receptors took a major leap forward with the cloning of the protein in 1996 (4) and its subsequent crystallization in 2016 (5). However, these structural insights have not answered many fundamental questions regarding how these proteins work. In PNAS, Alon et al. (6) present compelling information for the cloning of the sigma$_2$ receptor, completing the molecular characterization of this class of receptor and opening the door to more studies exploring mechanisms of action.

Hundreds of publications have addressed the functions of small molecules with affinity for the sigma$_2$ receptor, which has been implicated in cancer and neurodegenerative diseases (7–9), with a significant focus on the former. Through pharmacological studies, sigma$_2$ has been implicated in tumor biology (10) and has been proposed as a potential drug target in cancer therapy (11, 12), and sigma$_2$ radiotracers have been developed for tumor imaging (6). However, these associations were functionally based, with little molecular foundation. Identifying the gene for the sigma$_2$ receptor brings us much closer to unraveling fundamental questions about the pharmacology of this target. The association of sigma receptors with lipids and steroids goes back many years, and, in 2011, it was proposed that the sigma$_2$ receptor corresponded to a part of the progesterone receptor membrane component 1 (PGRMC1) complex (13). Although initial studies were encouraging, subsequent work questioned this identification (14–16). Most compelling was the fact that overexpression or knockdown of PGRMC1 failed to affect prototypic sigma$_2$ ligand binding.

The paper by Alon et al. (6) now resolves the question. Using classical affinity purification approaches, they isolated the sigma$_2$-binding site and identified it as the endoplasmic reticulum (ER)-resident membrane protein TMEM97, also known as MAC30. TMEM97 shows similar characteristics to the classical sigma$_2$ receptor-binding site, with the appropriate affinity and selectivity for a range of prototypic sigma$_2$ compounds. Mutagenesis studies then established the importance of two aspartate residues in the binding pocket. Although a significant step forward, identifying TMEM97 still leaves many questions. It has been associated with cholesterol homeostasis (17) and has been implicated in Niemann–Pick disease (18). Like sigma$_2$, TMEM97 is highly expressed in a range of cancers and has been associated with poor prognosis and even metastasis (19–23). However, much remains unknown about the functional role(s) of this protein and how the protein actually produces its actions. Major efforts in developing sigma$_2$ therapeutics have focused upon cancer, and, hopefully, these efforts will continue to shed light on the underlying mechanisms of TMEM97/sigma$_2$ actions.

Structurally, the sequence of TMEM97/sigma$_2$ predicts an integral membrane protein with an ER retention signal and four transmembrane domains with the N and C termini extending into the cytoplasm. The two aspartate residues important in binding are predicted to reside near the ER luminal surface of TMEM97/sigma$_2$. Initial predictions of the sigma$_1$ receptor structure from its sequence suggested two transmembrane domains with intracellular tails. However, the crystal structure of the protein was quite different, showing a

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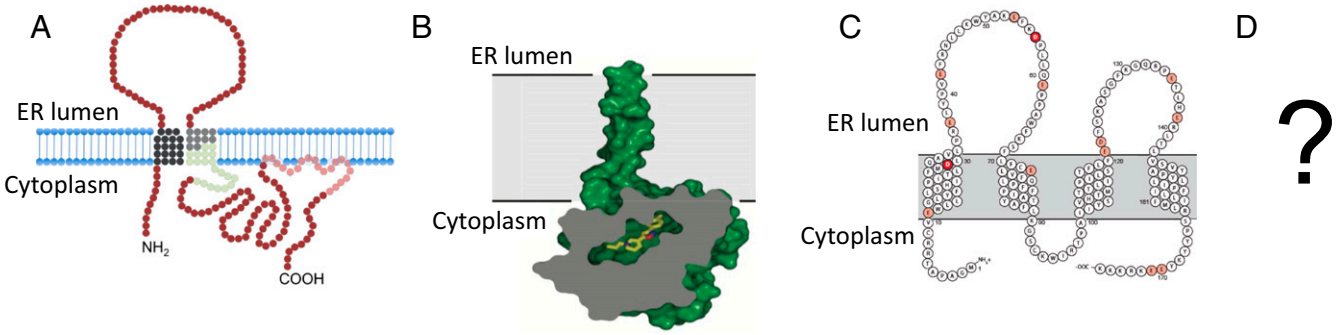

Fig. 1. Comparison of predicted topology and crystal structure of the sigma$_1$ and sigma$_2$ receptors. (*A*) Topology model of the sigma$_1$ receptor. Reproduced from Laurini et al. (26). (*B*) Space-fill model of the sigma$_1$ crystal structure with the bound ligand in yellow. Reproduced from Schmidt et al. (5). (*C*) Topology model of TMEM97/sigma$_2$ receptor. Reproduced from Alon et al. (6). (*D*) Question regarding crystal structure of TMEM97/sigma$_2$ receptor.

single transmembrane domain with a short extracellular N terminus and with most of the C terminus extending into the cytoplasm (5). It will be quite interesting to see if the crystal structure of TMEM97 corresponds to its prediction (Fig. 1). This next step is important in our endeavor to understand sigma$_2$ receptor function. Many have questioned the term "receptor" when referring to sigma. There is clearly a binding pocket with established structure–activity relationships. However, there is no endogenous ligand for either sigma receptor and no indication of a transduction system, with neither structure corresponding to any established receptor class. Presumably, as with sigma$_1$, sigma$_2$ ligand binding may lead to conformational changes that influence other, associated protein systems. The sigma$_1$ receptor shows an allosteric-like effect on the functions of proteins as diverse as the androgen receptor (24) and G protein-coupled receptors (25). It also interacts with a wide range of other classes of signaling proteins, receptors, and channels. Will sigma$_2$ receptors also have widespread activity? Are they primarily structural or modulatory? These questions are important, but remain to be answered.

Sigma receptors have had a long and nebulous history. Initially defined functionally, understanding them was limited by the selectivity of the ligands, which were often "dirty." However, extensive evidence suggested that they had the potential of yielding novel, important therapeutic agents in a range of disease areas, including cancer. The current cloning of the sigma$_2$ receptor is a major step forward in uncovering the molecular mechanisms responsible for sigma$_2$ ligand activity and brings us closer to understanding the true physiological role of these important proteins.

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