PROTEIN – CARBOHYDRATE INTERACTION

Because of their structural complexity and ubiquitous nature, carbohydrates play critical roles in many physiological as well as pathological cell functions. They are important adhesion receptors in cell–cell and cell–matrix interactions, and as such are absolutely necessary for leucocyte extravasation during inflammation and lymphocyte recirculation. Many microbes, including viruses, bacteria and their toxins, have evolved to bind cell surface carbohydrates, a binding that is a prerequisite for infection to occur. Likewise, carbohydrates are significant determinants of self/non-self, such that anti-carbohydrate antibodies may initiate graft rejection following transplantation between individuals of different ABO blood groups or between species. The fact that the carbohydrate phenotype changes with cell differentiation and tumour development may offer tumour biologists novel targets for immunotherapeutic strategies in the treatment of malignant diseases.

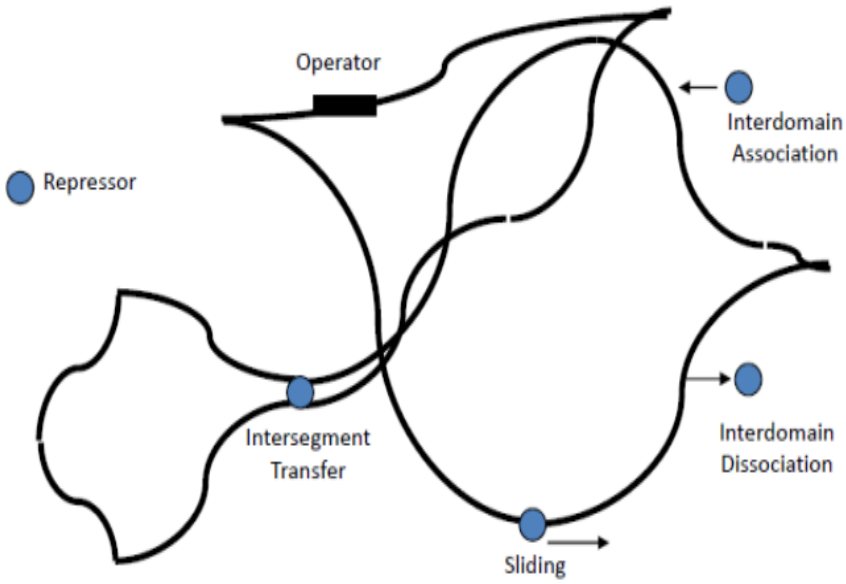
A well-known characteristic of protein–carbohydrate interactions is the low affinity of binding, usually in the millimolar range. These interactions are driven by a favourable enthalpy (heat is released), offset by the multiple contact points (hydrogen bonds, van der Waals' interactions and hydrophobic stacking) between the carbohydrate and the protein. In fact, recent studies have shown that in the binding site, polar–polar interactions are actually stronger than in the corresponding protein–protein interactions. One reason for this is shorter hydrogen bond distances, partly because hydrogen bonds involve charged groups as opposed to the polar– polar interactions in proteins, which mainly involve non-charged groups. In addition, highly organized hydrogen-binding atoms and a higher frequency of hydrogen bonds per unit area result in a closely spaced protein–carbohydrate interface. However, the favourable enthalpy is counteracted by an unfavourable entropy (degree of order) term that might stem from restricted carbohydrate flexibility. Enthalpy– entropy compensations are a characteristic of weak chemical interactions and are especially common in interactions involving water molecules, which readily form hydrogen bonds both with other water molecules as well as with hydrophilic surfaces of proteins and carbohydrates. As the human body has a water content of 70%, it is not surprising that water plays an essential role in both protein–protein and protein–carbohydrate interactions[9, 17]. Rather, it is likely that these interactions have evolved in the presence of water and, accordingly, water is an integrated part of the thermodynamics of the binding process[18]. Also, water molecules are specifically involved in the binding of proteins to carbohydrates by mediating ligation of the carbohydrate epitope to the binding cleft of the protein[19]. Accordingly, dehydration of the ligand and the receptor followed by rehydration of the formed complex between the two, as well as the role of water as a molecular mortar in protein–carbohydrate interactions, contributes to the overall binding affinity with enthalpy/entropy gains and penalties that seem to be individual for each ligand/receptor involved. Generally, complementary surfaces with well-matched polar interactions cause the escape of receptor/ligand-bound water molecules to the bulk, a reaction that favours the binding. In contrast, poorly matched surfaces result in incomplete desolvation of the molecules involved, and a low affinity binding[20]. In summary, protein–carbohydrate interactions constitute a unique structural-thermodynamic group where the individual changes in enthalpy and entropy might be substantial, yet the change in free energy of binding (ΔG°)

between a receptor and different ligands is relatively small. Consequently, increasing the affinity (i.e. increasing ΔG°) of specific protein–carbohydrate interactions remains a complicated task.

PROTEIN – DNA INTERACTIONS:

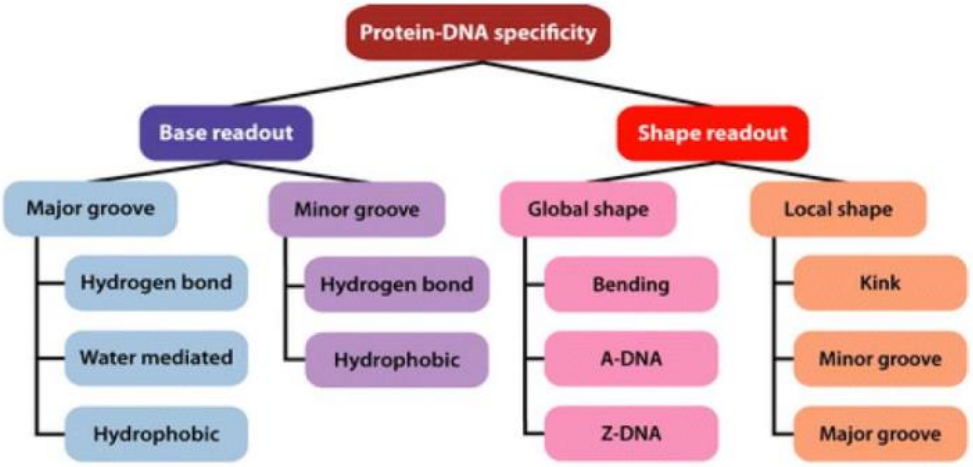
How do sequence-specific DNA binding proteins find their target sites in the genome?

How do sequence-specific DNA binding proteins find their target sites in the genome? Early work by Art Riggs and others have shown that bacterial transcription factors associate with their respective binding site in solution much faster than predicted according to macromolecular interaction kinetics. Binding to the specific target involves hydrogen bonding between the amino acid residues in the active site of the protein and base pairs in the minor or major grooves of the DNA. Non-specific interactions are electrostatic. Von Hippel and colleagues proposed that "the DNA cylinder can be viewed as an isopotential surface along which the protein can diffuse in a one-dimensional random walk". This sliding mechanism differs from chemical energy-dependent translocations by e.g. helicases and polymerases.



Spector, Annu. Rev. Biochem., 2003, von Hippel and Berg, JBC, 1989

Base Readout and Shape Readout

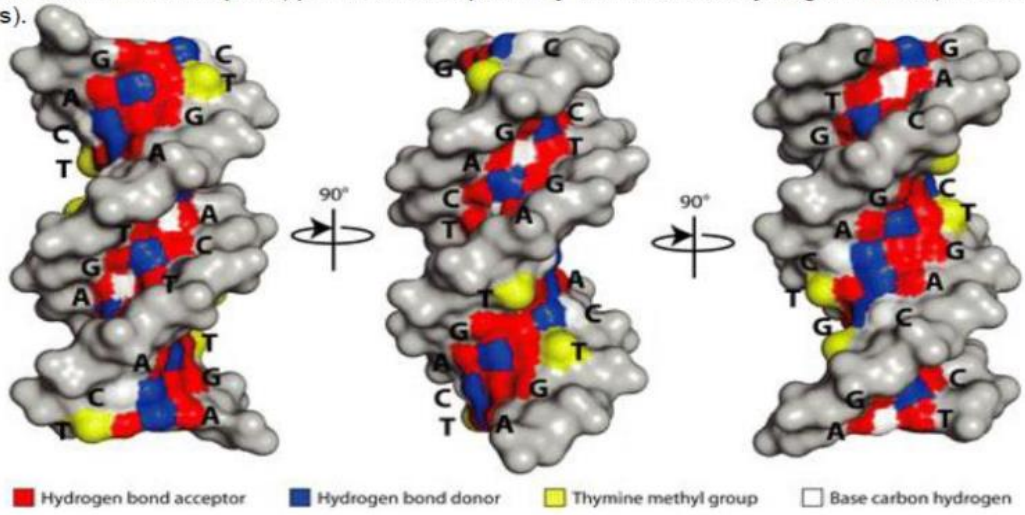


Rohs R, et al. 2010.
Annu. Rev. Biochem. 79:233–69

DNA binding proteins combine multiple readout mechanisms to achieve specificity. The topography of the human genome (assayed by hydroxyl radical cleavage pattern) is evolutionarily constrained and a better predictor of functional DNA elements than linear DNA. This suggests that DNA topology is an important contributor to the specificity of protein DNA interactions. Most of the more than 1500 solved protein/DNA structures reveal a form of bound DNA that deviates from the ideal B-form configuration.

Sequence-Specific DNA interactions

Proteins recognize chemical and conformational signatures of base pairs. Amino acid (AA) residues can interact with the edges of the base pairs in the major groove. These interactions involve hydrogen bonds, water mediated hydrogen bonds, or hydrophobic contacts. A:T versus T:A and C:G versus G:C are indistinguishable in the minor groove. Shown below are rotational views of the dodecamer $d(GACT)_3$ with hydrogen bond donors and acceptors, thymidine methyl groups, and base carbon hydrogen as indicated. Note that bidentate hydrogen bonds (two donors and two acceptors) provide more specificity than bifurcate hydrogen bonds (one donor and two acceptors).



Rohs R, et al. 2010.
Annu. Rev. Biochem. 79:233–69

Sequence-Specific Binding Proteins and DNA

1. Each BASE PAIR presents a unique constellation of sites for chemical interaction in the MAJOR GROOVE.

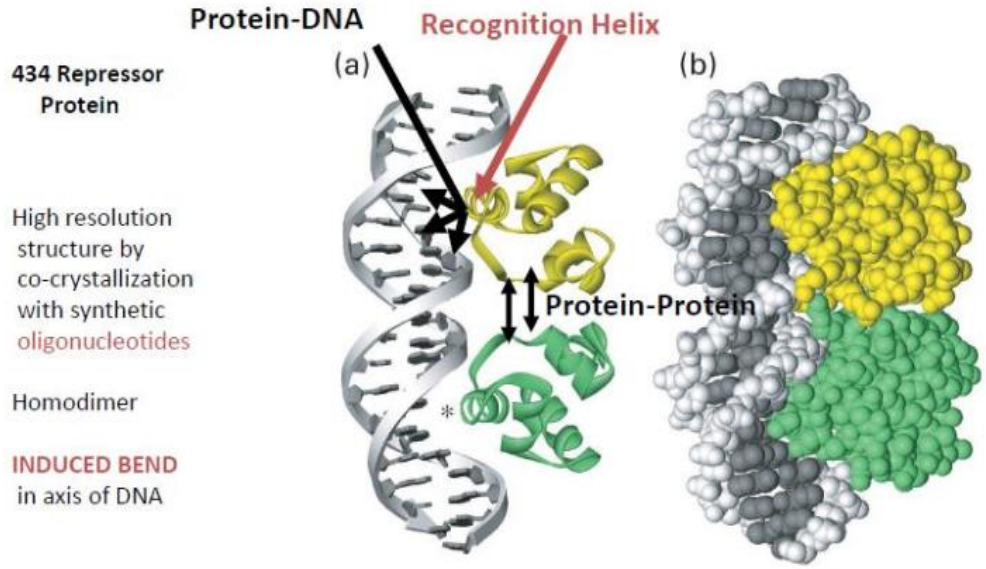
2. A RECOGNITION HELIX on the protein is positioned within the major groove.

3. The RECOGNITION HELIX participates in hydrogen bonds, Van der Waals interactions, and hydrophobic effect interactions with the base pairs. This is the basis of SEQUENCE RECOGNITION.

4. The most common (>80%) motifs for positioning the recognition helix are the HELIX-TURN-HELIX, the ZINC FINGER and LEUCINE ZIPPER. There are others.

5. Formation of a stable DNA-Protein complex is most often dependent upon additional non-covalent chemical interactions outside of the recognition helix-base pair contacts.

Helix-Turn-Helix



The helix turn helix (HTH) motif is the most commonly used secondary structure for specific DNA recognition. It was first characterized in prokaryotes. A recognition helix is positioned in the major groove and makes base specific contacts. A second helix stabilizes the recognition helix and is required for the proper positioning. Eukaryotic homeodomain proteins are characterized by a three helix bundle in which the 2nd and 3rd represent the HTH. The winged helix-turn-helix (wHTH) motif has an additional β-sheet that interacts with the minor groove to make additional DNA contacts.

Lodish Fig 7-28

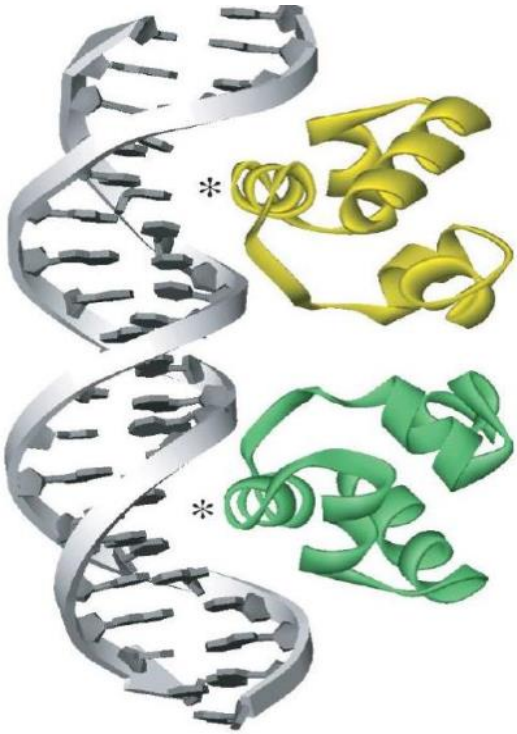
Homodimeric DNA Binding Proteins

Homodimeric proteins specifically recognize PALINDROMIC DNA sequences. Improved specificity

Binding energy additive, so interaction twice as strong as a monomer.

Some helix-turn-helix proteins are monomeric and do not have palindromic recognition sequences

5'
: :
A-T
C-G
A-T
: :
: :
: :
: :
T-A
G-C
T-A
: :
5'



PROTEIN – LIPID INTERACTIONS:

Biological membranes are complex, two-dimensional fluids formed from amphipathic lipid bilayers and a high density of proteins and carbohydrates. The organization of these components in live cells is heterogeneous, with order observed on a range of time and length scales. At a molecular level, it is the interaction potential between specific lipids and proteins that drives this organization, and to properly describe these interactions it is essential to understand the structure and dynamics of lipid–protein complexes. This has to some extent been observed using structural biology methods, where there are a growing number of membrane protein crystal structures in which a bound lipid has been identified. These crystallized lipid– protein complexes are thought to be representative of strong, long-lived lipid–protein interactions in the plasma membrane. In other systems, lipids exchange rapidly from sites along the perimeter of a protein into the bulk two-dimensional fluid at a rate of 10^7 s^{-1}. These two cases represent the range of dynamics that govern lipid protein interactions, but there is still a vast unexplored field of systems for which these dynamics have not yet been measured. One reason for this lack of data is that the interactions are very difficult to observe using traditional structural methods because of the nature of the lipid–protein environment and because of the fast dynamics of the interactions

Voltage-gated ion channels

Ion channels regulate charge transport across the plasma mem- brane. Gating and activity in these channels depend on a balance of ion concentration and electrical gradients

across the membrane. In voltage-gated potassium channels, a sensor domain on the outside of the channel induces structural rearrangements that open the pore and lower the energy cost for ions to cross through the membrane. This voltage-gating has been studied extensively, and there is growing evidence that the gating mechanism is lipid dependent. For example, inward rectifier potassium channels are thought to directly bind negatively charged PI(4,5)P2 lipids, and that the channel activity is proportional to the PI(4,5)P2 concentration. It has also been found that KvAP channels reconstituted in a bilayer with cationic lipids lacking a phosphodiester group lose their voltage-gated behavior. When phospholipids are added to the bilayer, the channels recover their voltage-gated activity with the largest enhancement resulting from anionic phospholipids. The hypothesized mechanism is that positively charged arginine side chains form hydrogen bonds with negatively charged phosphate groups in the lipid. This is consistent with lipid composition variations and directed point mutations, but to date there has been no direct probe of specific lipid head group–protein side chain interactions. Anionic phospholipids are also thought to regulate the activity of the mechanosensitive ion channels like MscL. It has been found that increasing concentrations of phosphatidylglycerol, phosphatidic acid, or cardiolipin increase calcein flux through the MscL channel, and that the effect is likely due to direct hydrogen bond formation rather than differences in spontaneous curvature .

Lipid-binding domains

Integral membrane proteins are not the only class of proteins that interact specifically with membrane lipids. Cytosolic proteins make use of a variety of structural motifs to anchor them to lipid membranes. For example, some proteins have amino acid sequences that encode for enzymatic attachment of lipid moieties that target the protein to a lipid bilayer. These are often used in signaling pathways to regulate localization of the protein to the plasma membrane, and have also been proposed to localize the protein to specific functional membrane domains. In spite of these observations, the physical interactions and the dynamic associations of these lipidated proteins are still poorly understood. For example, the lymphocyte cell kinase (Lck) protein is anchored to the membrane via two palmitoyl and a single myristoyl lipid modifica- tions that are thought to target the protein to cholesterol-rich membrane regions. However, there is evidence from biophys- ical studies that Lck partitions heterogeneously fluid domains in giant plasma membrane vesicles, which leaves open questions about the physical forces driving the organization of these proteins.

Another structural motif used to bind proteins to biological membranes is the inherent secondary and tertiary structures of the protein. Such lipid binding structural motifs include target- specific domains like the C1 domain that binds diacylglycerol, the pleckstrin homology (PH) domain that binds phosphoinositides like PIP2 and PIP3, and FYVE domains that bind PIP3. Other domains like PKC C2, annexin, BAR and F-BAR bind to anionic phospholipids non- specifically. While there is growing understanding of the protein–lipid binding kinetics, there are still core questions about secondary

interactions. For example, PH domain binding to phosphoinositides is strengthened by insertion of hydrophobic amino acids into the bilayer as well as non-specific interactions with negatively charged lipids. These interactions can be thought of as a two-dimensional analogue of co-solvent effects long studied in aqueous solutions. Central to the debate is how charge–charge interactions manifest themselves at the protein–lipid interface. Do anionic phospholipid head groups bind specifically to positively charged amino acid side chains, or is it better described as a non-specific co-solvent effect? Questions like this mirror those asked about the interaction of salts and osmolytes with proteins in aqueous solution. To date, however, there has been almost no direct investigation of this effect in biological membranes.

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