LESSON 5(b): HOW INFLUENZA ENTERS CELLS

The very first viral receptor ever discovered was the one that influenza uses: **sialic acid**. We learned in the last lesson that sialic acid is the very last sugar on many long chains. The chains can be made of nothing but sugar molecules, (such as glucose, or galactose), or the chain might include some amino acids. Chains that include both sugars and amino acids are called **glycoproteins**. "Glyco" means "sugar." The word glycoprotein is a very general word and can be used to describe very small structures or very large structures like the spikes we see on influenza. If a scientists were to list the basic parts of influenza, it would read like this: capsid, genome, lipid envelope, glycoproteins.

The glycoproteins we find sticking out of influenza's capsid are not all the same. If we look closely, we will see that there are two distinctly different shapes. Both shapes look a bit like clubs, with a "head" and a "stalk." The larger shape is called **hemagglutinin** (HE-mah-GLUE-tin-in) and the smaller one is called **neuraminidase** (noor-ah-MIN-i-dase).

Hemagglutinin (HA) gets its name from two words: "heme," meaning "blood," and "agglutinate," meaning "to clump." Why the reference to blood? Remember from lesson 3, we learned about a test that was developed in 1940, when they discovered that some viruses will cause red blood cells to clump. Red blood cells are covered with tiny glycoproteins that have sugar strings hanging off. The last sugar on the string is sialic acid so influenza is one of the viruses that will stick to red blood cells. This doesn't do the virus any good, though, as red blood cells don't have a nucleus. The influenza genome must enter a nucleus in order to replicate. So any viruses that stick to red blood cells will be "duds" that never reproduce. Cells in the trachea and lungs also have sialic acid sugars attached to them, and they, and they will be the cells that become infected.

The other glycoprotein, **neuraminidase** (NA), is like a little pair of scissors that only cuts one thing. It trims off the sialic acid sugar from the end of a chain. This will come in handy when the new virus tries to leave the cell. It doesn't want to be stuck to the cell it is coming out of. The NA trims off any sialic acids it touches, clearing the way for the virus to escape.

During the billions of times that viruses are replicated, small mistakes will be made. The result can be that the shape of HA or NA can change just a tiny bit. These changes may or may not affect the ability of the virus to cause infection. The successful changes are the ones that become permanent. Scientists have found (so far) 16 different shapes that HA can take. The first one ever discovered was called H1. The second was H2, and so on. The same holds true for NA. The first shape they discovered was called N1, then there was N2, N3, etc. up to N9. The famous flu pandemic of 1918 was a variety of influenza that had H1 and N1. (H1N1 still circulates today, but it is less dangerous than it was in 1918.) H2N2 caused the Asian flu outbreak of 1957. H5N1 caused the Bird Flu epidemic of 2004.

Influenza's capsid is surrounded by a lipid membrane that it took from the cell it came out of. The capsid itself is made of proteins. Embedded in the wall of the capsid are a few proton channels called M2. These channels will allow protons to come inside the capsid after the virus is inside an endosome in the cell.

Inside the capsid we find the viral RNA, broken into 8 pieces. At the end of each piece of RNA we find a little clump of proteins stuck to it. These proteins are copying machines that the RNA will need as soon as it gets into the host cell nucleus. There are also two small proteins that ride around with the genome. One is called NEP (Nuclear Exit Protein) which is like a copy of the password that all messenger RNAs need to get out of the cell nucleus. (Everything must pass through a heavily guarded exit pore.) The other small protein is NS1 (Non-Structural 1), and it prevents the cell from making its own RNAs, especially messenger RNAs that will be used to manufacture anti-viral "weapons" (such as Interferon).

Influenza is taken into cells by a process called "clathrin-mediated endocytosis." (Mediated means controlled, and endocytosis means "bringing things inside the cell.") **Clathrin** is a substance made of proteins that form a sort of scaffolding outside the endosome, like the temporary scaffolding on a building while it is being built. Little 3-armed proteins (triskelions) snap together on the under side of the membrane and work to pull the membrane down into a round shape. Another related protein curls around the top where it is getting narrow, and pinches it off like you pinch the end of a balloon after blowing it up. Then the endosome is cut free and is ready to travel.

Nothing moves on its own inside a cell. All movement is done by little "taxi" proteins called **motor proteins**. These amazing proteins have little "feet" that "walk" along "roads" made of long fibers called **microtubules**. Imagine a tightrope walker holding a house on his back as he slowly walks the rope-- that's pretty much what these motor proteins look like. They haul the endosome over to the nucleus.

While the endosome is riding along, the proton pumps in its membrane continue to pump, making the inside of the endosome more acidic. (About 5 on the pH scale for those of you who know about pH.) This lowering of pH is the trigger for HA to change its shape. (The technical term for this is a "conformational change." Scientists like long words.) The shape change will let a fusion protein pop out and stick into the cell membrane. Then the shape will change even more, and HA will begin to bend in half. When three HAs all do this together, the result is what they call "hair-pinning," meaning a very sharp bend in the membrane. The end result is that the membranes fuse together forming a hole from which the viral RNA can escape.

The pieces of viral RNA suddenly find themselves released right near the nucleus. They will be taken through the tiny pores that guard entry to the nucleus. Inside the nucleus they will begin to direct the manufacturing of viral proteins.

NOTE: Not all viruses must go to the nucleus. Some stay outside and use the ribosomes in the cytoplasm