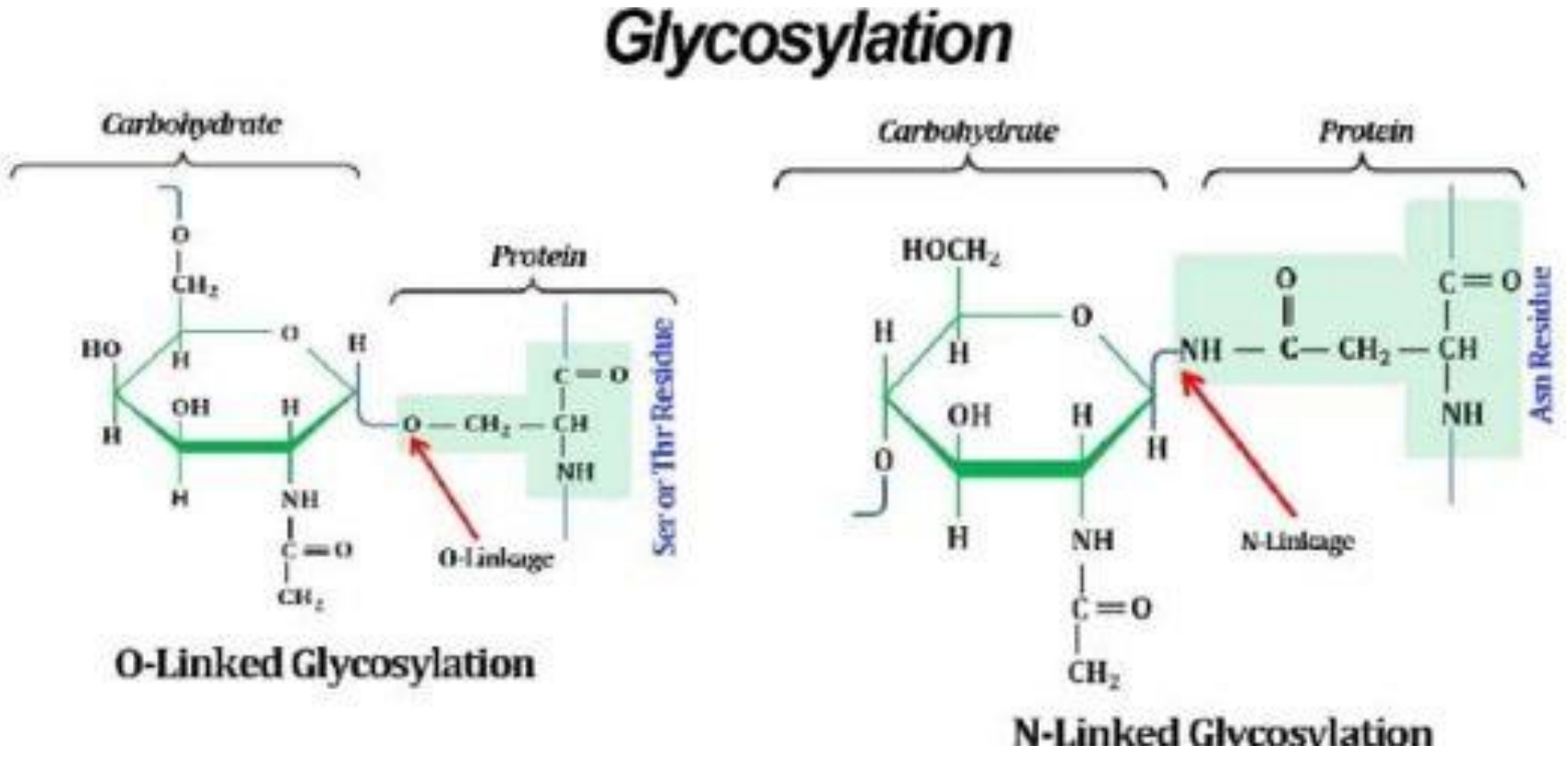
Glycosylation

- Co-, post- translational modification
- N-glycosylation:
  - the attachment of the sugar molecule (glycan) to a nitrogen atom of an asparagine (Asn) residue
  - Asn-X-Ser or Asn-X-Thr or rarely Asn-X-Cys
- O-glycosylation:
  - The attachment of a sugar molecule to an oxygen atom in an amino acid residue
  - Ser, Thr

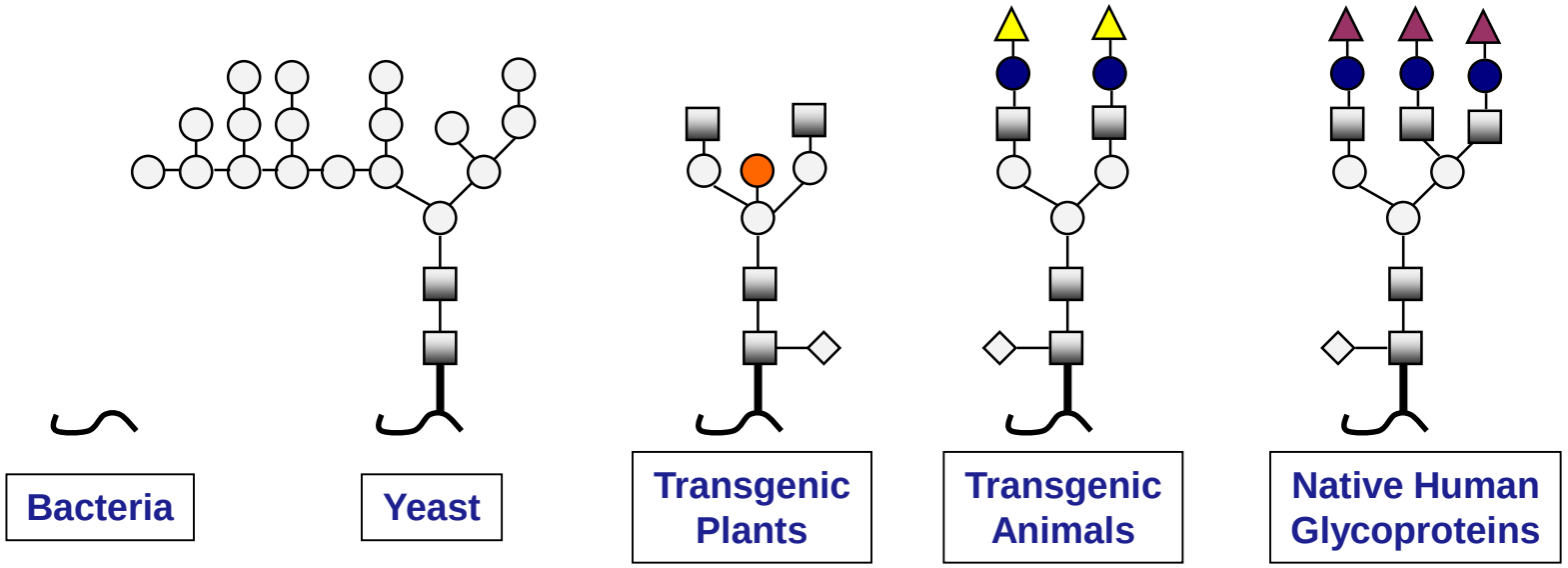
Glycosylation



Glycosylation

- Roles
  - Biological activity: EPO, β-interferon
  - Immunogenecity
  - Recognition
  - Stability: protection from protease attack
  - Solubility

Glycosylation



***N*-Acetylneuraminic acid** (**Neu5Ac** or **NANA**) is the predominant sialic acid (9-carbon α-keto acids) found in mammalian cells.

Host Cells

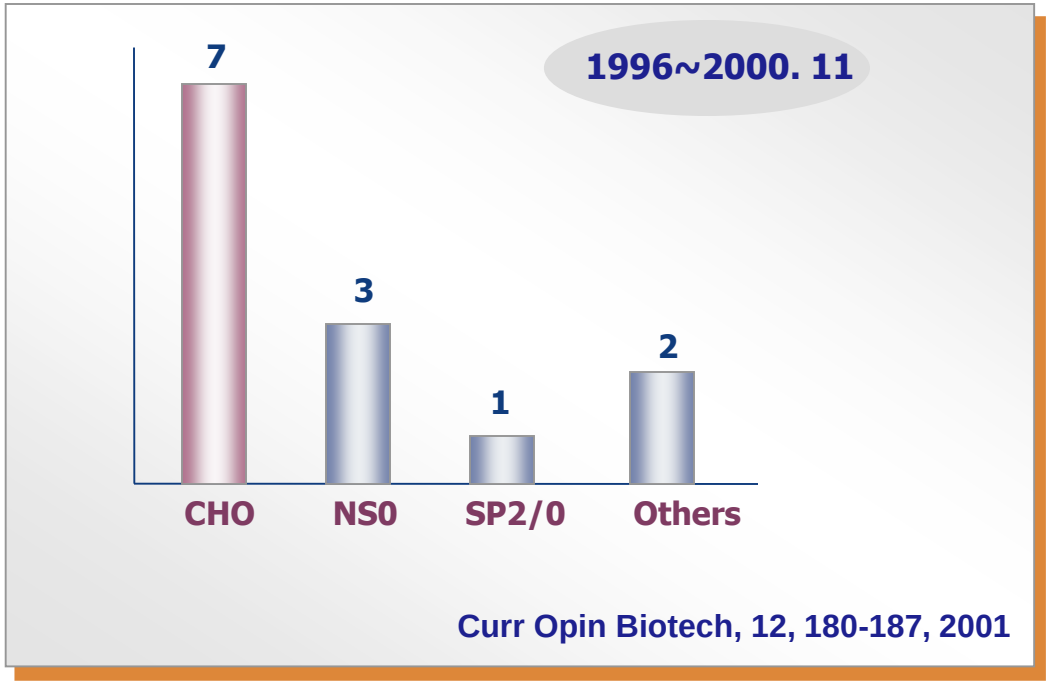
- CHO (Chinese Hamster Overy)
  - Most popularly used.
  - About 75% of commercial mammalian cell culture
  - Originally anchorage-dependent, however, nonanchorage-dependent cell lines were selected.
  - High product concentrations (up to 10 g/l protein product)
  - Effective secretion → simple recovery process.

Host Cells

- Other Cell Lines
  - Mouse myelomas (NSO and SP2/0)
  - Baby hamster kidney (BHK-21)
  - Human embryonic kidney (HEK-293)
  - Human retina-derived cell (PerC6)
- Human-based cell lines
  - Advantages
    - More authentic (humanlike) posttranslational processing
  - Disadvantages
    - Inadvertent human viral contamination necessitating multiple viral inactivation steps

Host Cells

- Recombinant therapeutics approved in the US using mammalian cell lines



CHO, NS0, PER.C6 …

Hybridoma Culture

- Hybridoma
  - Obtained by fusing lymphocytes with myeloma cells
  - Lymphocyte: producing antibodies
  - Myeloma: cancer cell proliferating indefinitely
- For the production of monoclonal antibodies (MAb's)

Formation of Hybridoma

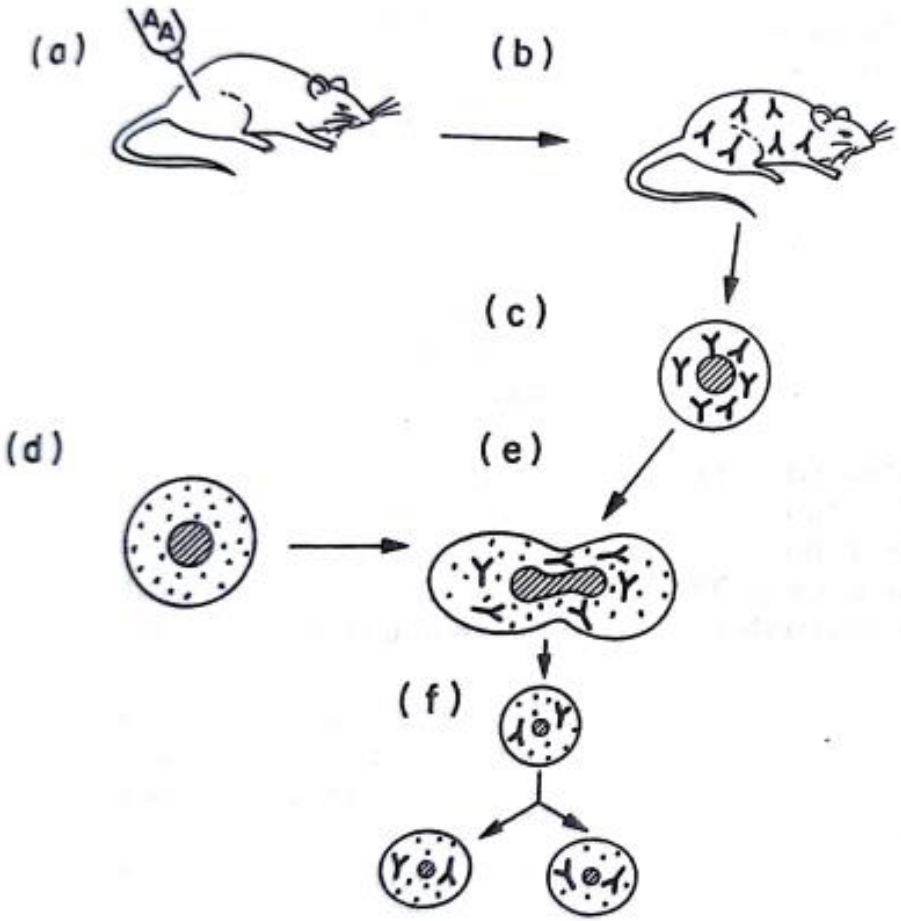


Figure 12.3. Formation of a hybridoma for making a monoclonal antibody. (a) Antigen is injected into a mouse; (b) lymphocytes in the mouse are activated to produce specific antibodies to the antigen; (c) lymphocytes are collected from the mouse; these lymphocytes grow poorly in tissue culture; (d) myeloma (cancer) cells growing in tissue culture are produced; (e) myeloma cells are fused with lymphocytes; (f) the hybrid cell grows well in tissue culture and make a single monoclonal antibody. Progeny are called *hybridomas* and can be propagated indefinitely.

Growth Media

- Growth medium contains serum (5% to 20%), inorganic salts, nitrogen sources, carbon and energy sources, vitamins, trace elements, growth factors, and buffers in water.
- Serum:
  - FBS (fetal bovine serum), CS (calf serum), HS (horse serum)
  - Serum contains amino acids, growth factors, vitamins, certain proteins, hormones, lipids, and minerals. (Table 12.2)
  - Exact composition is not known.

Major Functions of Serum

- To stimulate cell growth and other cell activities by hormones and growth factors
- To enhance cell attachment by certain proteins such as collagen and fibronectin
- To provide transport proteins carrying hormones, minerals, and lipids
- Table 12.2

Disadvantages of Serum

- Expensive ($100 to $500/L)
- Complications in cultivation and separation processes
- Possible contamination with viruses and mycoplasma
- Possible contamination by prion (mad cow disease)
- Foam generation in the serum-containing media
- Batch to batch variation

Growth Media

❑ **Serum-containing media**

- Basal medium with 5-10% FBS (v/v)
- Contamination
- in 1990s

❑ **Serum-free media**

- Devoid of Animal-derived materials
- Protein-free or Chemically defined media
- in 2000s

Growth Media

- MEM (Minimal Essential Medium)
  - Eagle's MEM
  - DMEM (Dulbecco's Modified Eagle's Medium)
- Rather complex media
  - Ham's F12, CMRL 1066, RPMI 1640
- Serum-free Media
  - 1:1 (v/v) mixture of DMEM (nutrient rich) and F12 (rich in trace elements and vitamins)
- Other more specialized media for specific cell lines