

- In 1955, Neils K. Jerne proposed the natural selection theory of antibody diversity. Jerne proposed that antibodies of all possible specificities are normally formed by the vertebrate host and delivered in small amounts into the blood. Any antigen that chances to enter the circulation reacts with the antibody specific for antigenic determinant. Jerne suggested that antigen acted as a selective carrier of antibody and carried the antibody to specialized cells capable of reproducing (synthesizing) this antibody. The specialized cells start producing this particular antibody in large amounts and pouring antibodies in the blood.
- In 1959, Burnet, Talmage and Lederberg proposed the widely accepted **clonal selection theory** (see Figure 5.3). They acknowledged Jerne's suggestion of the presence of pre-existing antibodies as the targets of antigen selection. They suggested that the "natural antibody" is located on the surface of lymphoid cell. The interaction of antigen with these antibodies (receptors) triggers (by some unknown mechanism) the signal for cellular differentiation and antibody production. The cell to which antigen is bound also starts proliferating to form clones of daughter cells possessing identical receptors and capable of identical immunological response. Thus, the antigen would serve to select and activate specifically the appropriate clonal precursor from a much larger population of lymphoid cells.

One problem remained, that of antibody diversity. It was well established that the body could generate antibody against almost any antigen that entered the body. One easy solution was to postulate the existence of a separate gene for each specific antibody. However, for this an organism would need to contain all the genetic information necessary to produce antibodies of about a million different specificities, even though each plasma cell would eventually produce antibodies of only a single specificity. This would need at least 500 times more total DNA in a single cell. As the structure of antibodies became known, it was noted that half of a light chain has a variable amino acid sequence and other half is constant. Similarly with heavy chains, a quarter of the chain is variable while the rest is constant. It was argued that if there are millions of genes ($\sim 10^8$ genes) of different antibody specificities present in the DNA, how is it possible to maintain this constancy of the sequence in the constant region?

- In 1965, W. Dreyer and J. Bennet proposed a solution to this problem. They suggested that the constant and variable regions of an immunoglobulin chain (heavy and light chains) are coded for by two separate genes, one gene for the variable region and the other for the constant region. They further clarified that diversity could be generated because hundreds or thousands of variable-region genes are present in the DNA of the cell, while one or a few genes code for the constant region of the immunoglobulin chain. At this point, the theory only had to account for multiple variable regions. The two genes for the single polypeptide theory were received with suspicion as it contradicted the then-accepted **one gene-one polypeptide principle**. Dreyer and Bennet suggested that these two genes must somehow come together at the DNA level to

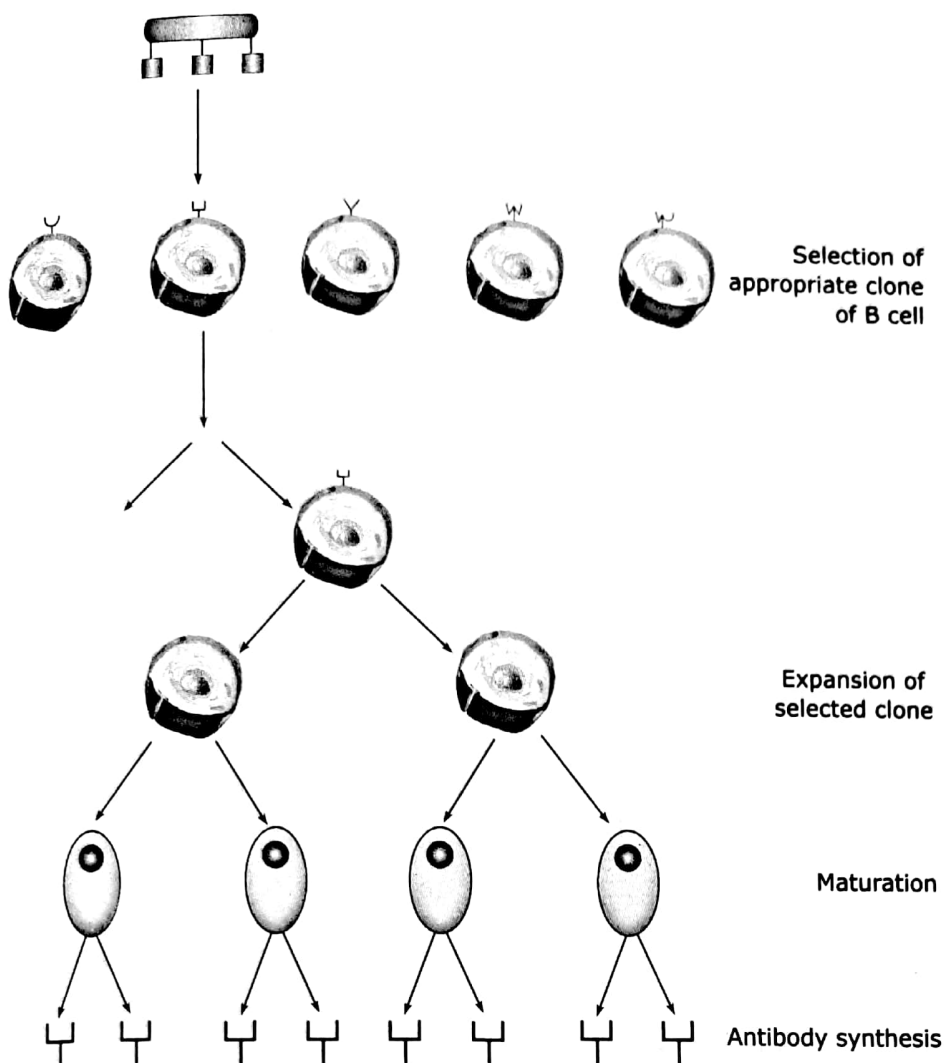


Figure 5.3

The clonal selection theory. Proposed by Lederberg and his colleagues, this theory established the mechanism for antibody diversity.

form a continuous message that is transcribed and translated into a single heavy or light chain.

- In 1978, S. Tonegawa and his co-worker working on genetically identical (syngenic) strain of mice isolated DNA from a 13-day-old embryo, and two different myeloma tumour cells—one which produced homogenous λ light chains (strain H2020) and one that produced κ light chains (strain MOPC321). They treated the DNA from these three sources with the same restriction enzyme EcoRI. These three types of DNA were electrophoresed, and then transferred to a nitrocellulose filter. The transferred DNA was denatured and then checked for sequences for the λ chain. The fully differentiated (λ -chain producer) myeloma cell DNA (H2020) gave four bands of 8.6 kb, 7.4 kb, 4.8 kb and 3.5 kb. The EcoRI digest of the fully differentiated κ -chain producer myeloma cell DNA (MOPC321) and embryonic cell DNA gave three bands (7.4 kb band was missing) (see Figure 5.4). These results suggested that during the development from embryonic state to a fully differentiated state, DNA undergoes some rearrangement. Further analysis was done with a probe that recognized specifically the constant region of λ gene (C λ probe) or the variable region of λ gene (V λ probe). These results clearly showed that the C λ probe hybridized with 8.6 kb and 7.4 kb fragments. V λ probe hybridized with 3.5 kb, 4.8 kb and 7.4 kb fragments.



Figure 5.4

Analysis of DNA fragments containing λ gene on agarose gel electrophoresis. (Cell by Brack et al. ©1978 by Elsevier Science & Technology Journals. Reproduced with permission of Elsevier Science & Technology Journals in the format Textbook via Copyright Clearance Center.)

This implied that the 8.6 kb fragment contains the constant region only of λ chain. The other two, 3.5 kb and 4.8 kb fragments, contain the variable region only of λ chain. And the 7.4 kb fragment (which is not present in the embryonic and mature κ -chain producer cells) contains both constant and variable regions on the same fragment.

Further analysis showed that the 7.4 kb fragment originates from a recombination (DNA rearrangement) event between 3.5 kb fragment and 8.6 kb fragment. These experiments demonstrated that Dreyer and Bennet's two-gene model, that is, the two gene-one polypeptide theory was true.

Subsequently researchers applied a similar approach together with the newly developed southern blotting techniques and demonstrated that Dreyer and Bennet's two-gene model (one gene coding the variable region and one gene encoding constant region) was true for both light and heavy chain genes.

Milestone 2.1—Clonal Selection Theory

Antibody production according to Ehrlich

In 1894, well in advance of his time as usual, the remarkable Paul Ehrlich proposed the side-chain theory of antibody production. Each cell would make a large variety of surface receptors which bound foreign antigens by complementary shape 'lock and key' fit. Exposure to antigen would provoke over-production of receptors (antibodies) which would then be shed into the circulation (figure M2.1.1).

Template theories

Ehrlich's hypothesis implied that antibodies were pre-formed prior to antigen exposure. However, this view

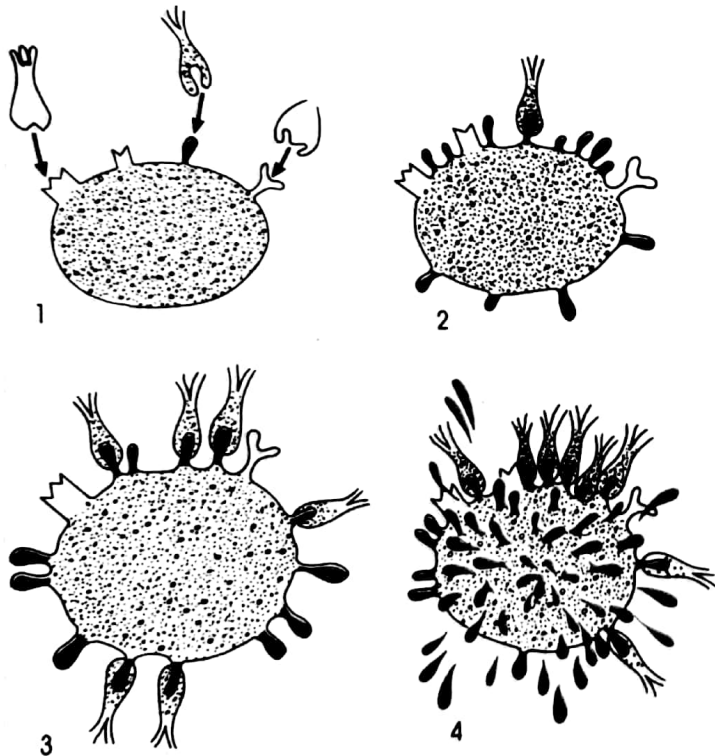


Figure M2.1.1. Ehrlich's side-chain theory of Ab production. (Reproduced from *Proceedings of the Royal Society B* (1900), 66, 424.)

was difficult to accept when later work showed that antibodies could be formed to almost any organic structure synthesized in the chemist's laboratory (e.g. azobenzene arsonate; figure 5.1) despite the fact that such molecules would never be encountered in the natural environment. Thus was born the idea that antibodies were synthesized by using the antigen as a template. Twenty years passed before this idea was 'blown out of the water' by the observation that after an antibody molecule is unfolded by guanidinium salts in the absence of antigen, it spontaneously refolds to regenerate its original specificity. It became clear that each antibody has a different amino acid sequence which governs its final folded shape and hence its ability to recognize antigen.

Selection theories

The wheel turns full circle and we once more live with the idea that since different antibodies must be encoded by separate genes, the information for making these antibodies must pre-exist in the host DNA. In 1955, Nils Jerne perceived that this could form the basis for a selective theory of antibody production. He suggested that the complete antibody repertoire is expressed at a low level and that when antigen enters the body, it selects its complementary antibody to form a complex which in some way provoked further synthesis of that particular antibody. But how?

Mac Burnet now brilliantly conceived of a cellular basis for this selection process. Let each lymphocyte be programmed to make its own singular antibody which is inserted like an Ehrlich 'side-chain' into its surface membrane. Antigen will now form the complex envisaged by Jerne, on the surface of the lymphocyte, and by triggering its activation and clonal proliferation, large amounts of the specific antibody will be synthesized (figure 2.11). Bow graciously to that soothsayer Ehrlich — he came so close in 1894!