How are Determination and Differentiation Interrelated?

Cell differentiation is a realization of the program, which was outlined in the course of determination and its first stage called predifferentiation. This stage was characterized by the cell's capacity for mitotic division, the absence of specific syntheses, and the presence of the endoplasmic reticulum, Golgi apparatus, and mitochondria. The differentiating cells produce significant amounts of specific substances and require the corresponding mechanism and sources of energy. At the next stage called protodifferentiation, the capacities of the cell for division are restricted and trace amounts of specific products are found, for example, insulin in Langerhans islet cells, i.e., this stage marks the beginning of specific syntheses, which characterize chemically a certain cell type. Thereafter, the cells reach the stage of differentiation per se, when the capacity for proliferation is fully lost and the concentration of a specific product sharply increases. The protein synthesis “machinery” starts to work at full power. The entire process is completed by terminal differentiation, which is characterized by a peak of synthesis of the specific product and “acquisition” of all characteristics inherent in the phenotype of a given cell (Fig. 1).

Since differentiated cells lose their capacity for reproduction, there is a cambial reserve in each tissue, which consists of the cells that retained their capacity for division. When a part of the differentiated cells die, their “pool” is replenished due to this reserve. This reserve includes also recently discovered stem cells.

FIG. 1. Phases of cell differentiation.

Abstract—A review of the data obtained by the author and his collaborators in studying tissue specific esterase of Drosophila males. Patterns were established for molecular-genetic regulation of synthesis of this isozyme.

Key words: esterase, isozymes, gene expression, Drosophila, ejaculatory duct.
They appear already in the embryos: embryonic stem cells (ES cells). During this period, ES cells are totipotent, i.e., they are capable of giving rise to any derivatives: ecto, endo-, or mesodermal. In adult tissues, stem cells are multipotent, i.e., they are capable of giving rise to many different cell types, but not to all of them. Quite unexpectedly, stem cells were found in the brain as well. There, as in other tissues, they are capable of repairing the defects related to the death of neurons. Nerve ES cells are capable of differentiating not only in the neural direction, but also in other directions and their range is not yet fully clear. They can be visualized on histological preparations using the immunohistochemical reaction for the stage specific protein nestin, which is synthesized in them. These cells pass through a number of stages in the course of differentiation. The first of them is the stage of progenitor cell, which is characterized by the synthesis of another protein, vimentin, and is capable of developing only in the neural direction, but can give rise to both neuroblasts and glioblasts. The next stage of restriction of the stem cell prospective potencies is the stage of precursor cell. It is capable of developing in only one direction: either into a neuron (neuroblasts specifically synthesizing β-tubulin) or into a glial cell (glioblast specifically synthesizing glial fibrillar acidic protein—GFAP).

**HOW CAN GENE ACTIVITY BE MONITORED IN THE DIFFERENTIATING CELL? WHAT ARE ISOZYMES?**

On what molecular-genetic events are determination and differentiation based? If the situation with differentiation is more or less clear, determination still remains a largely enigmatic process in this respect. The questions related to this problem are better being considered on a specific experimental model. It is evident that for studying determination and differentiation at the molecular level, it is necessary to have: (1) a genetically controlled product, (2) a gene encoding this product, and (3) a homogenous system of cells, which synthesize this product, in order to exclude admixtures of cells differentiating in another direction and synthesizing other products.

The possibility to monitor indirectly the functioning of genes according to the appearance of their products first appeared in 1958 after the discovery of isozymes by Markert and Moller and evidence of their genetic nature provided by Shaw, geneticist from Texas. Markert and Moller made their discovery accidentally. They studied the sequence of appearance of different protein fraction in ontogenesis of mammals using electrophoresis in starch gel and decided to identify fractions with certain enzymes using new, at that time, methods of histochemical staining. Imagine their surprise, when having stained one of electrophoregrams for lactate dehydrogenase (LDH), they found that positive reaction was recorded in five protein fractions, rather than one. These fractions were called isoenzymes (isozymes). Initially, isozymes were defined as any multiple fractions of the same enzyme visualized in the same organism (Markert and Moller, 1959). However, Shaw soon found a mutant for LDH and proved the genetic mechanism of appearance of five fractions of this enzyme. It turned out that LDH, a tetramer, consists of two types of subunits, M and H, encoded by individual genes, which are located in different chromosomes. M subunit predominates in the somatic muscle (hence the name M from muscles), while H subunit in the heart (H from heart). These subunits can be united in a tetrameric molecule in any combination, so that five combinations can be formed: HHHH, HHHM, HHMM, HMMM, and MMMM (Markert and Ursprung, 1971). Since LDH subunits somewhat differ in amino acid composition and, hence, in electric charge, tetrameric molecules that differ in the composition of subunits are characterized by different electrophoretic mobility, which is expressed in the appearance of five LDH isozymes. LDH is a sufficiently interesting enzyme, since it plays an important role in glycolysis and catalyzes a reversible reaction:

\[
\begin{align*}
\text{CH}_3\text{CH}–\text{COOH} + \text{NAD} – \text{CH}_3\text{C}–\text{COOH} + \text{NADH} + \text{H}^+.
\end{align*}
\]

Lactic acid Pyruvic acid

Naturally, LDH isozymes were first studied for expression in ontogenesis assuming that the activity of corresponding genes can be assessed by the distribution of activities of these isozymes. We will see that this not fully the case, but studied of the dynamics of LDH isozyme spectrum in ontogenesis made it possible to reveal the temporal and spatial specificity of this pattern. The slow isozyme LDH-5 predominates in the embryonic tissues of mice. It may be explained physiologically by that LDH-5 can intensely function under anaerobic conditions characteristic, for example, for embryonic tissues or skeletal muscle. Its activity is the highest at those concentrations of pyruvate (substrate) that inhibit LDH-1. Evidently, the predominance of LDH-5 should ensure rapid transformation of pyruvate into lactate and, hence, lead to oxygen debt under anaerobic conditions.

However, in some skeletal muscles of mammals, for example, in the soleus muscle, the same distribution of LDH isozymes was registered, as in the cardiac muscle. This is explained by that such muscles contain predominantly red fibers, whose main physiological role con-