REPRESSION AND DEREPRESSION OF LACTATE DEHYDROGENASE LOCI IN MICE DURING DEVELOPMENT

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Changes in the lactate dehydrogenase (LD) spectrum were studied by ultramicroelectrophoresis during development of mice: ripe ovum–zygote–blastocyst–embryo–oocyte–ripe ovum. In the cells of the embryo until the fifth day of development only H subunits of LD were found. The appearance of M subunits indicates derepression of the LD-A locus. On the eighth day of embryonic development the LD-B locus was derepressed. In the course of oogenesis the content of M subunits was reduced in the spectrum of the oocytes, a result of progressive repression of the LD-A locus. The dictyotene stage of the prophase of meiosis was characterized by active synthesis of H subunits and a gradual decrease in the synthesis of M subunits. The LD spectrum of the follicular cells throughout the stages of dictyotene was of the M type.

KEY WORDS: lactate dehydrogenase isozymes; oogenesis; ontogeny.

The study of repression and derepression of the mammalian genome is not only of great theoretical interest for the understanding of normal development, but also of practical importance for the solution of problems in the treatment and prevention of hereditary diseases and congenital deformities and the elucidation of the mechanisms of regeneration of organs, of cellular immunity, and of tissue incompatibility.

The enzyme lactate dehydrogenase (LD; L-lactate: NAD-oxyreductase, EC 1.1.1.27) is a convenient model with which to study the function of the loci during differentiation. It is present in the organs as five isozymes, each of which is a tetramer consisting of two types of polypeptide chains (H and M), coded by two nonallelic autosomal genes (LD-B and LD-A) [8].

The object of this investigation was to study alternation of the phases of activity of the LD loci in the cycle: ripe ovum–zygotes–embryo–oocyte–ripe ovum.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice (Stolbovaya nursery). Females with accurately dated pregnancy were obtained [2]. Before implantation the embryos were removed by flushing out the cavities of the oviducts and uterus with 0.15 M NaCl solution in 0.05 M phosphate buffer, pH 7.3. The embryos at the stage of implantation and early organogenesis were removed as follows: The wall of the uterus was cut longitudinally, the embryonic sacs were transferred to physiological saline, the decidua was carefully incised, the embryo was extracted, and it was separated from the cells of the embryonic sac by means of a microneedle.

To obtain follicles the ovaries were treated with a 0.25% solution of warm trypsin (5-10 min), washed with physiological saline, then transferred to warm Tyrode–Mascona solution (without Ca and Mg ions) for 10-15 min [3]. To obtain oocytes, the follicles were transferred into a 0.10% solution of hyaluronidase, made up in Tyrode–Mascona solution, and then punctured. Oocytes from which all follicular cells had been removed were washed several times with a 20% sucrose solution in the isolation medium [9].

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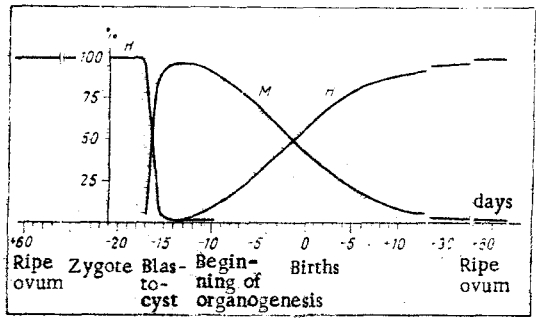

Fig. 1. Activity of LD loci in developmental cycle of mouse: ovum–zygote–blastocyst–embryo–oocyte–ovum (schematic). Abscissa, age (in days); ordinate, content of subunits (in %).

The supernatants were subjected to ultramicroelectrophoresis in polyacrylamide gel [1]. The gels after electrophoresis were incubated to detect LD isozymes [4]. The gels were scanned on a microdensitometer with automatic writer and the content of isozyme in the spectrum was determined from the area of the peaks. The relative quantities of H and M subunits were calculated, assuming a tetramer structure of the enzyme. The theoretically expected distribution of isozymes in the spectrum was obtained on the basis of binomial combination of polypeptide chains into tetramers [7].

EXPERIMENTAL RESULTS

Analysis of the early stages of mouse embryonic development showed that the fertilized ovum (zygote) contains only the LD-1 isozyme (H subunits). This isozyme persists in the cells of the embryo until the implantation stage (fifth day) (Fig. 1). The M subunits of LD were found for the first time in the cells of the embryo during the period of implantation, a fact which evidently reflects derepression of the LD-A locus in the genome of the embryo. The gene responsible for synthesis of H subunits was derepressed a little later (8th-10th day).

The genome in the fertilized mammalian ovum is considered to be inactive and derepression of the embryonic genes is considered to begin at the stage of two blastomeres [5, 6]. The information necessary in the initial stages of development of the embryo is evidently contained in the cytoplasm of the ovum (mRNA, protein) and is the result of gene activity in oogenesis. Development of female germinative cells is an example of cell differentiation which occupies a large part of ontogeny. These experiments showed that the LD spectrum in the oocytes of newborn mice (the dictyotene stage of prophase of meiosis) is characterized by predominance of anodal fractions of the enzyme, which distinguishes it sharply from the LD spectra in the other tissues studied at that stage (Table 1). The fraction of H subunits in the spectrum of the newborn mice is 0.576. This proportion of H subunits is found in the heart and kidney cells of adult animals only (0.578 and 0.577, respectively). The fraction of H subunits in the LD spectra of the testes of newborn mice is lower, namely 0.486. The predominance of H subunits in the LD spectra of the ovaries evidently reflects the more active work of the locus controlling the synthesis of subunits of this type.

The study of the LD spectra in the riper oocytes at the dictyotene stage showed predominance of homotetramers (LD-1 and LD-5); the distribution of the isozymes differed in the largest follicles of the ovaries of mice aged 1 and 2 months and differed significantly from the expected distribution (Table 1). These deviations can be attributed to the fact that the LD spectrum of the follicles at a certain stage of development is the combined spectrum of oocytes and follicular cells. Only the isozyme LD-1 was found in the LD spectrum of the ripe unfertilized ovum. Presumably the anodal part of the LD spectrum of whole follicles of mice aged 1 and 2 months contains the set of LD isozymes in the oocyte and the cathodal part of the spectrum contains the set of isozymes of the follicular cells.

The dynamics of the LD spectra during the course of oogenesis suggests that initially both LD loci are derepressed in the oocytes. As the oocytes ripen, a progressive decrease in the synthesis of M subunits is observed. This leads to their complete absence in the ripe oocyte (complete repression of the LD-A locus) (Fig. 1).

In the course of oogenesis information for the synthesis of H subunits (mRNA) which is used in early embryogenesis thus accumulates. After the fifth day of embryonic development derepression of the LD-A locus coding the synthesis of M subunits is established. Derepression of the second LD-B locus is observed on the

TABLE 1. Changes in LD Spectrum during Developmental Cycle of Mice: Ripe Ovum–Zygote–Blastocyst–Embryo–Oocyte–Ripe Ovum

Age, days	Material	E or T	LD spectrum, %				
			H_4	H_3M	H_2M_2	HM_3	M_4
	Ovum	E	100,0	0	0	0	0
—20	Zygote	E	100,0	0	0	0	0
—16,8	Embryo	E T	4,5 0	0 0,4	0 3,1	11,1 23,2	84,5 74,5
—15,4	Embryo	E T	4,9 0,1	0,9 0,4	3,1 5,4	12,3 29,2	79,0 65,7
—14	Embryo	E T	1,3 0	0 0	0 0,6	5,2 9,6	93,6 90,5
—13	Embryo	E T	0 0	0 0,4	1,5 3,0	24,1 22,0	74,5 75,9
0	Whole ovary	E T	23,7 11,0	22,8 32,4	23,8 35,9	19,6 17,5	10,1 3,2
+30	Large follicles	E T	14,8 1,19	7,1 9,6	12,9 29,4	25,9 39,6	39,5 20,2
+60	Large follicles	E	7,7	27,6	37,4	22,3	5,0
+60	Ovum	E	100,0	0	0	0	0

Legend. E) Experimental values of relative content of LD isozymes; T) theoretically calculated values of relative content of LD isozymes. 0) Births; –) days before birth; +) days after birth.

8th-10th day of embryonic life. In the course of morphogenesis the activity of each locus becomes established in the various organs. In the postnatal life of the animal one locus is gradually repressed: the LD-B locus in the hepatocytes and the LD-A locus in the ova, for example.

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