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NUCLEOLUS-ORGANIZING REGIONS OF CHROMOSOMES IN EARLY EMBRYOGENESIS OF LABORATORY MICE

E. L. Patkin and A. V. Sorokin

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Both transcription and translation of several genetic loci take place in mammals starting from the 2-4 blastomere stage [1]. In particular, biochemical methods have reliably demonstrated the presence of rRNA synthesis starting from the 4 blastomere stage [8, 9]. However these methods cannot establish whether all chromosomes carrying nucleolus-organizing regions (NOR) participate in transcription in initial embryogenesis. In 1975 Goodpasture and Bloom [5] developed a method of selective demonstration of NOR of chromosomes which, as was subsequently shown [10, 12, 14], reveals precisely those NOR which functioned in the preceding interphase as organizers of the nucleolus, i.e., staining with silver reflects the function of ribosomal genes and not simply their presence. A method of silver staining was used in [4, 6] to study the time of onset of NOR activity in early mouse embyrogenesis but, as our own observations have shown, the methods used by these workers to prepare their specimens and the method of staining with silver are not optimal.

The object of this investigation was to determine the conditions for most complete demonstration of NOR in early mouse embryogenesis and to study changes in the number of NOR starting from the first cleavage division and until the 10th day of development.

EXPERIMENTAL METHOD

Experiments were carried out on CBA × C57B1 mice obtained from the "Rappolovo" nursery. The mice (females) were given an intraperitoneal injection of 0.2 ml of 0.02% colchicine 1-1.5 h before they were killed on the 1st, 2nd, 3rd, 4th, and 10th days of development, after which specimens were prepared by the method adopted in the writers' laboratory: The embryos were placed in 0.9% sodium citrate for 4-15 min, fixed in a mixture of methanol with glacial acetic acid (3:1), and then macerated on a slide in a mixture of 75% acetic acid and methyl alcohol (1:1). For selective staining with silver a modified method of Howell and Black [7] was used. There were two solutions: A 2% solution of gelatin to which formic acid was added (1 ml to 50 ml gelatin) and a 50% solution of silver nitrate, purified beforehand by Khachaturov's method [4]. Both solutions were made up in deionized water. One drop of gelatin and two drops of silver were applied to the freshly prepared specimens, a coverslip was placed over them, and they were incubated in a humid chamber for 5-15 min at 60°C until they were stained a golden brown color. The specimens were then vigorously rinsed in running water and dried

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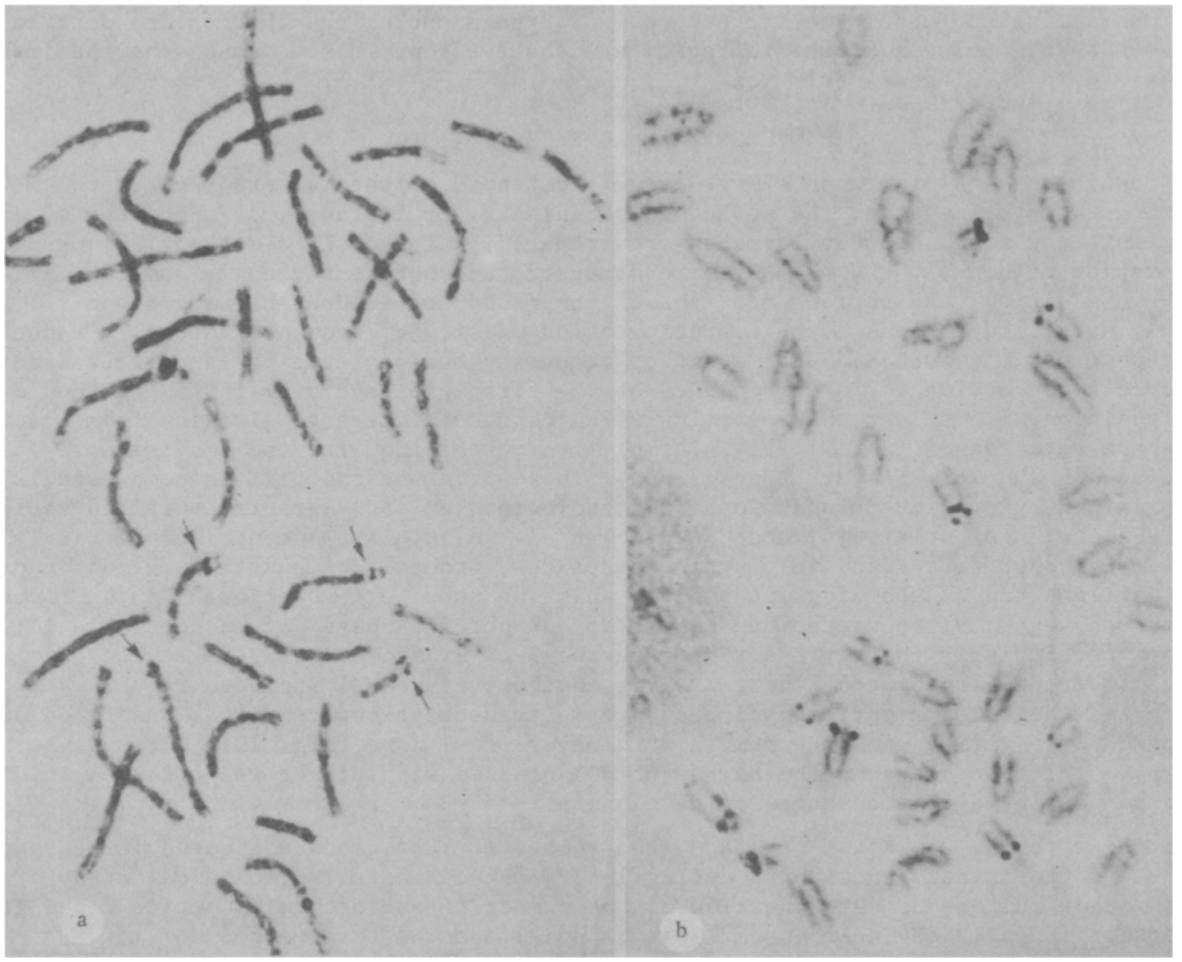

Fig. 1. NOR in chromosomes of mouse embryos: a) 2 blastomere stage; b) blastocyst stage (64 cells). Stained with silver. Ocular 12, objective 100.

TABLE 1. Time Course of Discovery of NOR in Chromosomes during Initial Embryogenesis of Mice with Selective Staining with Silver

Stage of development	Number of					
		metaphases		NOR per metaphase		
	embryos	total	with NOR	minimal	maximal	average
First cleavage division	70	46				
Second cleavage division	100	110	50	1	7	1,5±0,3
4—7 Blastomeres	25	35	33	1	8	3,5±0,3
8—16 »	25	42	40	4	10	6,6±0,3
17—24 Blastomeres	20	34	34	4	8	6,9±0,2
25—35 Blastomeres	15	30	30	4	8	7,0±0,1
38—60 »	10	35	35	4	10	7,0±0,2
Over 60 blastomeres	8	32	32	4	10	8,1±0,1
10th Day of development	15	150	150	1	8	4,8±0,3

in air. On examination under the microscope, interphase nuclei and chromosomes were colored yellow and NOR and nucleoli brown. To facilitate observation, in some cases the specimens were counterstained in 2% Giemsa's solution.

EXPERIMENTAL RESULTS

The results of staining with silver largely depended on the factors such as the method of preparing the specimens and the method of staining. For instance, if specimens of embryos in preimplantation stages of development were prepared by Tarkowski's method [13] or by the double fixation method [1, 2], the NOR were demonstrated much less clearly and in smaller numbers. In addition, the various versions of the method of Goodpasture and Bloom [5], which were mainly used, led to considerable precipitation of silver, and this naturally reduced the resolving power of the method. The method of preparing and staining the specimens described above proved to be optimal. In chromosomes of the first cleavage division no NOR could be found by staining with silver, but starting with the second cleavage division from 1 to 7 NOR per metaphase were found (Table 1; Fig. 1a, b), i.e., starting with the 2 blastomere stage, genes coding 18S and 28S RNA were transcribed. These observations fill in some details of results obtained by previous biochemical, autoradiographic, and ultrastructural investigations [8, 11]. Unlike Engel et al. [4] and Hansmann et al. [6], in the second cleavage division not one but seven NOR were found in certain metaphases, probably because the method of preparing the specimens and of staining with silver which we used were superior. In the course of subsequent cleavage divisions the mean number of NOR per metaphase increased (Table 1); the number rose particularly sharply starting with the 8-16 blastomere stage. At the late blastocyst stage (over 60 blastomeres) there was yet another very small increase in this value, i.e., at that period there was a corresponding change in transcription activity. The range of the number of NOR also varied with the number of cleavage divisions (Fig. 1b). It must be pointed out that a definite decrease in the number of NOR stained with silver was observed in chromosomes at the postimplantation stages of development.

Starting with the 2 blastomere stage, the number of rRNA genes activated in the genome is the same as the number functioning during later embryogenesis. Cytological study of rRNA synthesis with simultaneous determination of the number of synthetically active genes and their location in individual chromosomes can be undertaken by this method.

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