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during Mouse Oocyte Growth

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Mouse oocytes can be classified according to their chromatin organization and the presence [surrounded nucleolus (SN) oocytes] or absence [nonsurrounded nucleolus (NSN) oocytes] of a ring of Hoechst-positive chromatin around the nucleolus. Following fertilization only SN oocytes are able to develop beyond the two-cell stage. These studies indicate a correlation between SN and NSN chromatin organization and the developmental competence of the female gamete, which may depend on gene expression. In the present study, we have used the HSP70.1Luc transgene (murine HSP70.1 promoter + reporter gene firefly luciferase) to analyze gene expression in oocytes isolated from ovaries of 2-day- to 13-week-old females. Luciferase was assayed on oocytes after classification as SN or NSN type. Our data show that SN oocytes always exhibit a higher level of luciferase activity, demonstrating a higher gene expression in this category. Only after meiotic resumption, metaphase II oocytes derived from NSN or SN oocytes acquire the same level of transgene expression. We suggest that the limited availability of transcripts and corresponding proteins, excluded from the cytoplasm until GVBD in NSN oocytes, could explain why these oocytes have a lower ability to sustain embryonic development beyond the two-cell stage at which major zygotic transcription occurs. With this study we have furthered our knowledge of epigenetic regulation of gene expression in oogenesis.

Key Words: **oogenesis; chromatin organization; transgene; heat shock protein; luciferase.**

INTRODUCTION

During oocyte growth, gene expression is highly active in order to accumulate the maternal products that are required, after fertilization, to sustain the first steps of embryonic development (reviewed by Telford, 1990). Several experiments have shown that maternal factors are able to modify developmental ability (Latham *et al.*, 1991) and gene expression in the zygote (Kothary *et al.*, 1992; Chastant *et al.*, 1996). Although it is as yet unclear which are the factors playing a role in the regulation of gene expression in early development, it is likely that their quality and quantity are determinant for the survival of the newly formed zygote.

A recent study has shown that germinal vesicle (GV) oocytes isolated from the antral compartment of the mouse ovary display a meiotic and developmental competence which is correlated to their chromatin organization (Zuccotti *et al.*, 1998). Within the antral compartment of the mouse ovary (Mattson and Albertini, 1990; Debey *et al.*, 1993, Zuccotti *et al.*, 1995) and in that of other mammals (Lefevre *et al.*, 1989; Mandl, 1962; Crozet *et al.*, 1981), including human (Parvenov *et al.*, 1986), two types of oocytes differing in their chromatin organization have been described: (1) oocytes with a Hoechst-positive ring of chromatin surrounding the nucleolus and a compacted, thread-like nuclear chromatin [surrounded nucleolus (SN) oocytes)] and (2) oocytes without a ring of chromatin surrounding the nucleolus and a diffused nuclear chromatin

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[nonsurrounded nucleolus (NSN) oocytes]. Of these two types, SN oocytes are those that, after *in vitro* culture and insemination with spermatozoa, develop *in vitro* beyond the two-cell stage, whereas NSN oocytes terminate their development by the second cell cycle (Zuccotti *et al.*, 1998).

These observations have established a strong link between nuclear structure and the oocyte cellular activity. It remains to characterize more precisely which components of the cellular activity are modified and how these transformations influence the oocyte's capability for embryonic development. A crucial point is that chromatin structure can directly change transcriptional activity or gene expression (Kadonaga, 1998). However, only a few studies have investigated this relationship during oocyte growth using microinjected plasmid hybrid genes, which are not integrated in the chromatin (Sorensen and Wassarman, 1976; Martines-Salas *et al.*, 1989; Wickramasinghe *et al.*, 1991; Bevilacqua *et al.*, 1992). In this study, we address the question of whether the changes in chromatin organization described during oocyte growth and maturation (Zuccotti *et al.*, 1995) have an influence on the regulation of gene activity in SN and NSN oocytes. We have used a transgenic model created with a construct coupling the murine promoter HSP70.1 to the reporter gene firefly luciferase (Christians *et al.*, 1995) which allows the detection of gene expression in very few oocytes thanks to the very high sensitivity of the luciferase assay (Christians and Renard, 1996). Here we present the results of our study following analysis of luciferase activity in ovulated metaphase II or ovarian oocytes, the latter isolated at different stages of growth (from primordial to antral follicles) and classified according to their SN or NSN chromatin organization.

MATERIALS AND METHODS

Reagents

All reagents were purchased from Sigma Co., unless otherwise stated.

Transgenic Lines

Transgenic HSP70.1Luc mouse lines were established by microinjecting zygotes according to conventional procedure (Hogan *et al.*, 1986). The transgene is a *Sca*I-linearized Bluescript plasmid bearing the murine HSP70.1 promoter coupled to the firefly luciferase cDNA (for detail see Christians *et al.*, 1995). All the mice used in this study were homozygous from different lines named NLF 1, 14, and 30. The genetic background of transgenic animals is the result of successive crossings between hybrids C57BL/6 × CBA.

Superovulation of Transgenic Females

According to the type of experiment, some of the transgenic females were either superovulated or not. They received the following treatments: (1) intraperitoneal (ip) injection of 7.5 IU PMSG (pregnant mare serum gonadotropin), 48 h before collection of oocytes; and (2) ip injection of 7.5 IU PMSG followed after 48 h by ip injection of human chorionic gonadotropin (hCG) and sacrifice 15 h later.

Oocyte Isolation and Culture

Transgenic females of various ages (3 days to 13 weeks) were killed by cervical dislocation and dissected to collect ovaries and/or oviducts. Oocytes of different size (from 10- to 20-μm-diameter primordial follicles to 70- to 80-μm-diameter antral follicles) were isolated from ovaries as previously described (Zuccotti *et al.*, 1995). Mature oocytes (metaphase II stage, MII) were obtained either following *in vitro* culture of antral oocytes or from the oviducts following ovulation. Different types of media were used: PB1 or M2 for oocyte collection (see Wood *et al.* in Monk, 1987), M16 (see Wood *et al.* in Monk, 1987), α-MEM (Life Technologies, Milan, Italy), Kasaï, or KSOM (Kasaï *et al.*, 1978; Lawitts and Biggers, 1993) for oocyte culture. Media were prepared in ultrapure MilliQ water (Millipore). In some experiments, inhibitors of germinal vesicle breakdown (GVBD), such as dibutyryl cyclic adenosine monophosphate (dbcAMP) (100 μg/ml) and isobutyl methyl xanthine (IBMX) (stock solution in pure DMSO 45 μg/ml) (Alexandre *et al.*, 1988), or inhibitors of transcription such as α-amanitin (100 μg/ml) (Kidder *et al.*, 1985) were added to culture media.

Oocyte Observation and Classification

Oocytes were first classified according to their size and then separately stained by incubation in M2 medium containing 0.05 μg/ml Hoechst 33342 (Zuccotti *et al.*, 1998). Classification based on SN or NSN type of chromatin organization was performed according to Zuccotti *et al.* (1995).

Luciferase Assay

Luciferase assay has been described elsewhere (Christians *et al.*, 1995). Single or groups of oocytes were stored in 0.5-ml Eppendorf-type tubes in 50 μl lysis buffer (Nguyen *et al.*, 1988) and stored at −20°C for not longer than 1 week. In order to measure luciferase activity, samples were thawed, transferred to an assay tube, and diluted 1:1 with distilled water. Luciferase assays were performed with a luminometer (Lumat LB 9501, Berthold, Germany) that automatically added an assay mixture (100 μl of lysis buffer with 0.8 mM ATP and 0.3 mM firefly luciferase (Ref.: L9504) to the assay tube. The light emission was integrated for the first 10 s. Background level for each series of samples to be assayed was measured under the same conditions with lysis buffer (blank), and 110% of the value obtained was subtracted from every measurement displayed by the luminometer (in order to exclude all nonresponder oocytes). Oocytes (10–20 μm in diameter) from primordial follicles were dissected from ovaries of newborn females and the luciferase assay was performed on groups of 40 pooled oocytes. Follicular cells remaining attached to the oocytes could not interfere with oocyte analysis, as groups of several hundred follicle cells gave only background values of luciferase activity. For oocytes of larger diameter, measurements were done either on groups of a maximum of 15 oocytes or on single oocytes.

Statistical Analysis

Statistical analyses were performed using two-tailed parametric (*t* Student) and nonparametric tests [Kruskal–Wallis (*H*), Dunn (*Q*),

TABLE 1
Mean Luciferase Activity during Oogenesis

Females HSP70.1Luc		Oocyte size [μm] (volume [μm^3])			
Line (NLF No.)	Age (weeks)	10–15 (523.6–4188.8)	≤50 (65449.8)	50–60 (65449.8–113097.3)	>60 (113097.3)
1	Newborn	2 ± 0.4[a] (400)[b]	—	—	—
	2–13	—	871 ± 35.7 (223)	986 ± 129 (33)	1878 ± 159 (44)
14	6–13	—	—	—	3015 ± 185 (38)
30	6–13	—	269 ± 32 (3)	401 ± 61 (10)	591 ± 43 (61)

[a] Mean luciferase activity ± standard error (RLUs/oocyte) of the mean.
[b] Number of oocytes measured in each category.

and Mann–Whitney (*U*)]. Spearman rank correlation was also used for estimating the correlation coefficient between classes.

RESULTS

Transgene Expression during Oocyte Growth

Five transgenic lines were created with a construct coupling the murine HSP70.1 promoter to the reporter gene firefly luciferase and both homozygous males and females were obtained from three of five lines. Transgene expression was detected in oocytes collected from homozygous females belonging to these three different HSP70.1Luc transgenic lines. Table 1 shows the level of transgene expression in oocytes obtained by puncturing ovaries. Oocytes were classified in four groups according to their size (volume): 10–20 μm (523.6–4188.8 μm^3), <50 μm (65449.8 μm^3), 50–60 μm (65449.8–113097.3 μm^3), and >60 μm (113097.3 μm^3) in diameter. While luciferase activity is barely detectable in primordial oocytes (2 ± 0.4 RLUs per oocyte), the transgene is highly expressed as soon as oocyte growth has begun (50 μm in diameter). The level of luciferase activity increased with oocyte growth. Correlation between oocyte growth and luciferase activity was estimated using Spearman rank correlation ($r = 0.983$; $P < 0.001$).

Transgene Expression in Growing SN or NSN Oocytes

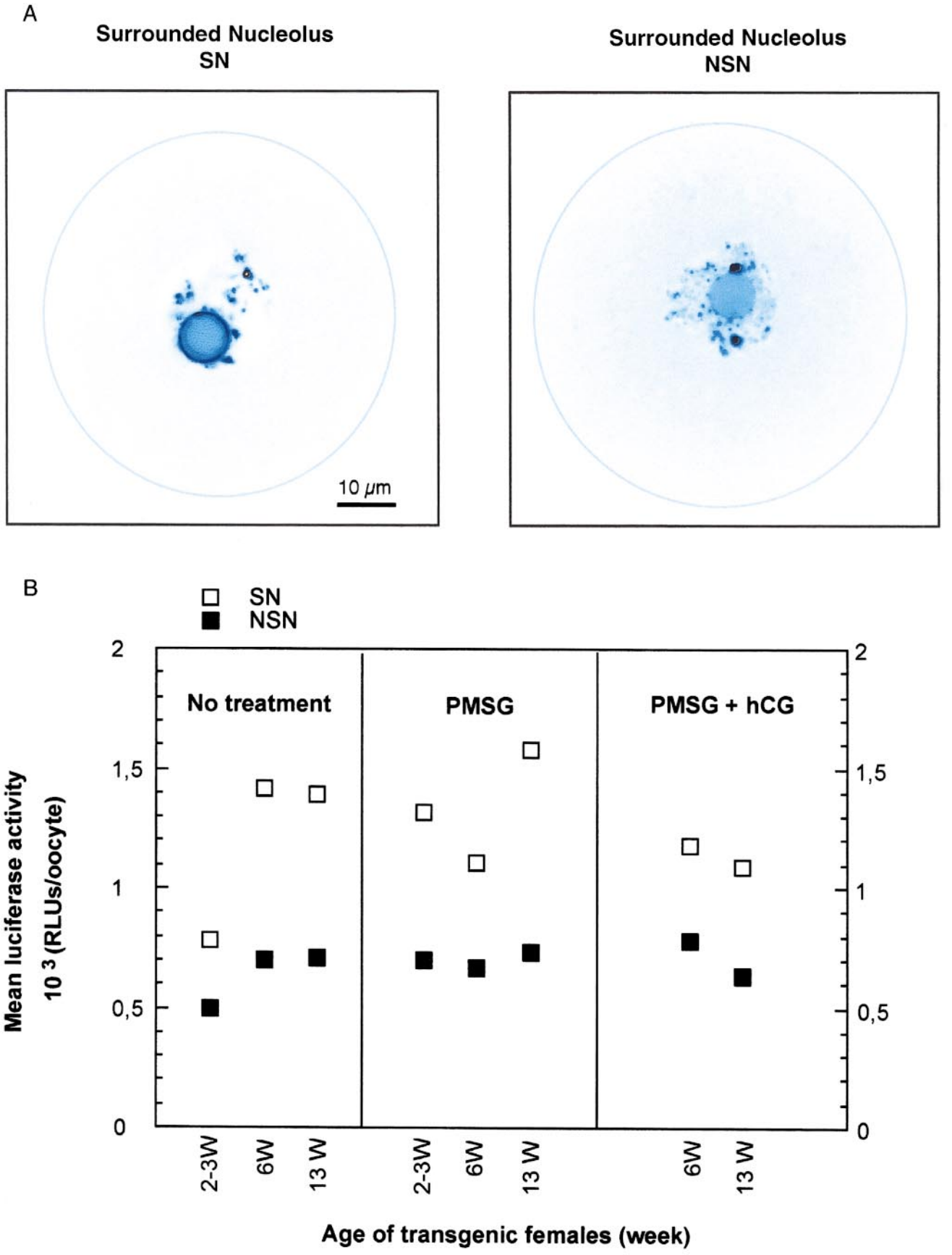
Following isolation, female germ cells were first classified according to their size and their chromatin organization in SN or NSN oocytes, then they (a maximum of 15 and an average of 12 oocytes per sample) were assayed for luciferase activity (Figs. 1A and 1B).

We analyzed oocytes of different sizes collected from 2- to 3-, 6-, and 13-week-old females treated or not with hormones. Figure 1B shows the data obtained for 60- to 65-μm oocytes. Independent of the age of the female, and of whether females were treated or not with PMSG, SN oocytes exhibited a significantly higher level of luciferase activity compared with oocytes of the NSN group (*U* test: $P = 0.000$ for untreated females, $P = 0.001$ for PMSG-treated females). Similar results were obtained with oocytes collected after PMSG + hCG injections. Ratio of luciferase activity measured in SN/NSN growing oocytes isolated from PMSG-treated females was about 2 in the different diametrical classes (50–60, 60–65, 70–80 μm in diameter) (null hypothesis was ratio = 1; Student *t* test, $P < 0.05$) and did not change significantly (Student *t* test, $P >> 0.05$).

Analysis of Luciferase Activity in GV and MII Oocytes

Transgene expression was analyzed in GV antral oocytes and in MII oocytes either obtained *in vitro* (following culture of antral oocytes) or collected from the oviducts of superovulated females at different time intervals after hCG injection (Fig. 2A). A significantly higher luciferase activity was found in MII oocytes (obtained either *in vitro* or *in vivo*) compared with GV oocytes isolated from the antral compartment (Student *t* test, $P = 0.000$) (Fig. 2A). This difference between MII and GV antral oocytes was observed in all

FIG. 1. Chromatin organization and level of transgene expression in SN and NSN oocytes. (A) Fluorescence micrographs of mouse HSP70.1Luc oocyte nuclear organization after TO-PRO 3 staining. Oocytes from postpubertal transgenic females were collected by puncturing ovaries, fixed and permeabilized according to Kubiak *et al.* (1992), and stained by incubation in TOPRO-3 (0.001 mM) (Molecular Probes). Samples were analyzed with a Leika confocal microscope. Bar, 10 μm. (B) After classification in SN or NSN category, pooled or single oocytes (60–65 μm in diameter) were assayed for luciferase activity. Mean luciferase activity was calculated for eight different groups according to the age of the female (2–3, 6, or 13 weeks) and the superovulation treatment (no treatment, PMSG injection, or PMSG and hCG injections). Mean size of each group is 20 oocytes collected from at least two different females.


A
Surrounded Nucleolus
SN
Surrounded Nucleolus
NSN
10 μm
B
SN
NSN
No treatment
PMSG
PMSG + hCG
Mean luciferase activity
10 3 (RLUs/oocyte)
2
1,5
1
0,5
0
2-3W
6W
13 W
Age of transgenic females (week)

the HSP70.1Luc lines analyzed (data not shown). The difference in luciferase activity found between MII oocytes obtained *in vitro* (15-h culture) or *in vivo* (isolated from the oviducts 15, 24, or 42 h post-hCG) was not significant (Student *t* test, $P > 0.05$). A significantly higher luciferase activity was instead detected when comparing MII oocytes obtained *in vitro* following 24 h culture with oocytes obtained *in vivo* 15, 24, or 42 h after hCG injection (Student *t* test, $P < 0.05$). Therefore, the passage from GV to MII (either *in vitro* or *in vivo*) acts in the same way on luciferase activity, whereas the time elapsed after reaching MII has a different effect on oocytes obtained *in vitro* or *in vivo*.

In order to investigate the reason why luciferase activity increased during meiotic resumption, several experiments were designed and their results are summarized in Fig. 2B. To exclude late changes in transcriptional activity during this period, GV oocytes were incubated in medium including α-amanitin, an inhibitor of RNA polymerase II. It is known that inhibition of transcription does not hamper meiosis resumption in mice (reviewed by Motlik, 1989). Despite oocytes being cultured in α-amanitin, their luciferase activity increased to the same extent of that detected in MII oocytes obtained in the absence of inhibitor. In contrast, when GVBD was inhibited on purpose by either dbcAMP or IBMX, luciferase activity remained low ($P < 0.001$), reinforcing the idea that the passage of oocytes from GV to MII through GBVD is the responsible event and not the time spent in culture. The type of medium does not seem to play a major role in this variation in luciferase activity.

Influence of NSN or SN Chromatin Organization on Transgene Expression in MII Oocytes

Levels of luciferase activity were analyzed in *in vitro*-matured oocytes obtained from classified SN or NSN oocytes following PMSG or PMSG + hCG injections (Fig. 3). Ratio of antral SN/NSN oocytes under various hormonal conditions changed (data not shown) following a pattern similar to that described earlier (Zuccotti *et al.*, 1995). Also, as previously shown (Zuccotti *et al.*, 1998), SN oocytes have a better meiotic competence than NSN oocytes (data not shown). Prior to meiotic resumption, SN oocytes collected from females treated with PMSG exhibited a twofold higher level of luciferase activity than NSN oocytes (*Q* test after Kruskal–Wallis ANOVA and *U* test, $P << 0.05$). Following 15 h of *in vitro* culture and resumption of meiosis, luciferase activity remained higher in MII oocytes derived from SN compared to those derived from NSN oocytes, but the difference was no longer significant (*Q* test after Kruskal–Wallis ANOVA and *U* test, $P > 0.05$). Similarly, MII oocytes derived from NSN or SN oocytes isolated following PMSG + hCG injections did not show a significantly different luciferase activity (*Q* test after Kruskall–Wallis ANOVA and *U* test, $P > 0.05$). Variation of luciferase activity from GV to MII oocytes following PMSG treatment showed a significant increase in both SN (Student *t* test, $P < 0.05$) and NSN oocytes (Student *t* test, $P < 0.05$), although this increase was higher for NSN than for SN oocytes (Fig. 3B).

DISCUSSION

The data presented in this paper show that the HSP70.1Luc hybrid gene can be used as a marker to follow transcriptional activity and gene expression during oogenesis. Reporter genes have been widely used in transgenic mice lines to analyze transcriptional activity of regulatory regions of known and unknown genes (Cui *et al.*, 1994; Allen *et al*, 1988). The different transgenic lines created with the HSP70.1Luc hybrid gene have been involved in various studies on zygotic gene expression in general and in regulation of heat shock gene in particular. A first set of preliminary analyses revealed that high level of luciferase activity detected in ovaries was due to HSP70.1Luc transgene expression in oocytes (Christians and Renard, 1996). This was observed in all transgenic lines created with this hybrid gene. To our knowledge there are only two previous studies reporting transgene expression during oogenesis: the ZP3Luc hybrid gene (Lira *et al.*, 1990) and, more recently, the cytomegalovirus immediate early enhancer–chicken β-actin (CAG) LacZ hybrid gene (Sakai and Miyazaki, 1997). In fact, such an expression was unexpected for HSP70.1Luc as previous studies have shown that endogenous HSP 70 proteins are not synthesized at normal temperature during oogenesis (Bensaude *et al.*, 1983; Curci *et al.*, 1991) and also because RT-PCR experiments have indicated that endogenous HSP70.1 gene is transcribed at a very low level (Christians *et al.*, 1995). However, HSP70.1Luc expression could be explained by the large amounts of mHSF1 (Christians *et al.*, 1997) and Sp1 (Worrad *et al.*, 1994) stored in the germinal vesicle. Both of them have binding sites in the HSP70.1 promoter (two for HSF and three/four for Sp1; Hunt and Calderwood, 1990) and therefore they can stimulate its transcriptional activity. In addition replacement of HSP70.1 coding sequence by a reporter gene prevents negative feedback, which has been proved to play an important part in the regulation of HSP genes (Shi *et al*, 1998). For example, Bevilacqua *et al.* (1992, 1993) have shown that microinjected HSP68LacZ construct can be transiently transcribed in growing oocytes. With these observations in mind, we set out (1) to investigate transcriptional activity during oocyte growth using as a marker HSP70.1 Luc expression and (2) to find out whether different oocyte chromatin configurations previously described in mouse oocytes (Matson and Albertini, 1990; Debey *et al.*, 1993; Zuccotti *et al.*, 1995, 1998) could affect such an expression.

In nongrowing oocytes (primordial follicles) luciferase activity was not detected even if they were presumably transcriptionally active (Schultz, 1986), but when oocytes

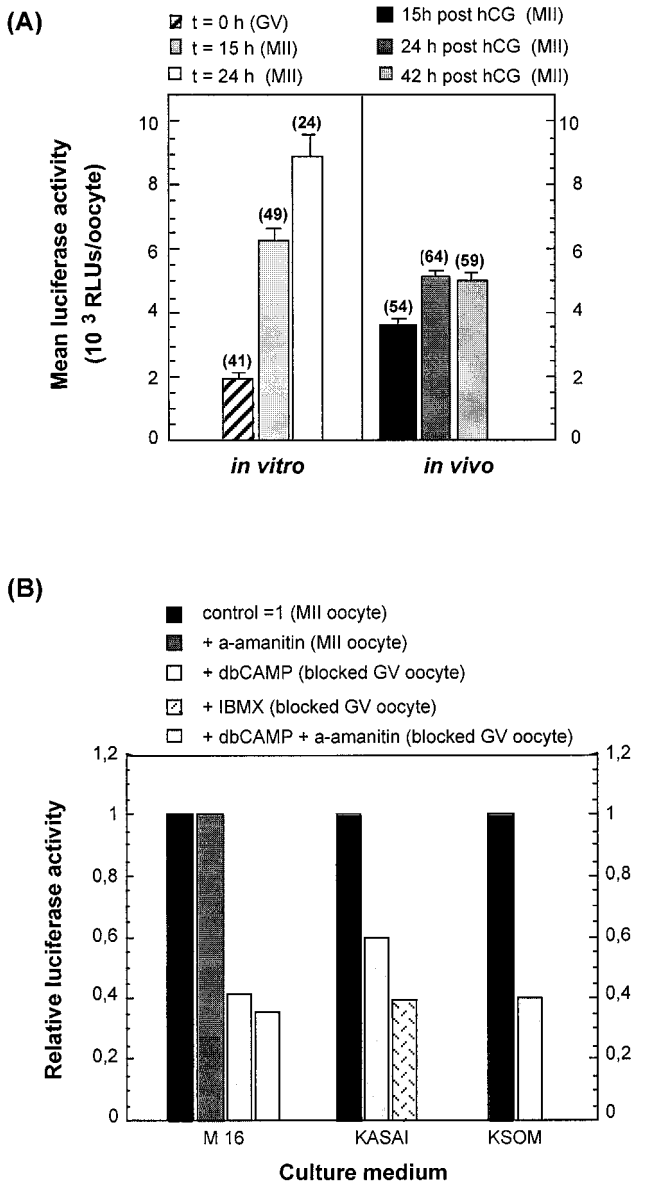


FIG. 2. Effect of meiosis resumption on transgene expression. (A) Transgene expression in oocytes matured either *in vitro* or *in vivo*. Oocytes collected from ovaries of postpubertal females were cultured *in vitro* during various times (t = 0, 15, 24 h) in M16 medium before luciferase activity was assayed. *In vivo* matured oocytes were recovered from oviducts of superovulated females and directly lysed to perform luciferase activity measurement. Timing is indicated in hours post-hCG injection. Number of single oocytes analyzed to calculate the mean luciferase is shown in parentheses. Error bars denote error of the mean. (B) Luciferase activity in oocytes cultured for 15 h in different media containing inhibitors. Transgene expression in treated oocytes is plotted relative to control oocytes. At least 20 oocytes were analyzed for each point. Chromatin organization (GV or MII) is indicated for each group.

(40–50 μm in diameter) were isolated from ovaries of 2-week-old females (when it is known that oocyte growth has started) transcription of HSP70.1 transgene was detected. HSP70.1 promoter is therefore efficiently recruited by transcriptional machinery activated during oocyte growth with a correlation between oocyte size and the level of luciferase activity. The status of gene expression has been suspected to be different in oocytes exhibiting a nucleolus either surrounded or not by a ring of chromatin (Debey *et al.*, 1993; Vautier *et al.*, 1994). In this study we show that the level of luciferase activity is indeed systematically and significantly higher in SN oocytes than in NSN oocytes, independent of the age of the transgenic females, the treatment of females before collection (no treatment, PMSG, or PMSG + hCG), or the size of the oocytes (Fig. 1). Our data clearly indicate that these two groups of oocytes are not only morphologically but also functionally different. We have earlier demonstrated that, following *in vitro* resumption of meiosis and *in vitro* fertilization, SN oocytes are capable of development beyond the two-cell stage, whereas NSN oocytes never develop further (Zuccotti *et al.*, 1998). Therefore, we can hypothesize that the difference observed here in gene expression for SN and NSN oocytes may explain their different developmental competence.

It was beyond the scope of our study to explain which factors or parameters could explain these differences. However, it is worth mentioning the features presented in Vautier *et al.* (1994): they report that chromatin in SN oocytes is on the whole more condensed than in NSN oocytes; thus we should have had a lower expression in the former category than in the latter as it is assumed that gene expression is less active in condensed chromatin. When they analyzed several nuclear antigens which can be involved in RNA processing and transport (i.e., 43B1N, p-120, and SC-35), they showed that a larger number of such granules is present in NSN oocyte but with a different size and/or distribution (Vautier *et al.*, 1994; Borsuk *et al.*, 1996). These authors suggest that this different labeling in SN and NSN oocytes may be correlated with different nuclear activities. Our data corroborate this hypothesis and may help to indicate which pattern of nuclear antigens (e.g., larger and less numerous p-120 granules observed in SN oocytes) may be indicative of a more efficient gene expression activity.

As we were interested in analyzing gene expression in oocytes in relation to chromatin organization, we investigated transgene expression after meiosis resumption and completion, while major changes occur in chromatin organization toward its progressive condensation into chromosomes. Strikingly, MII oocytes exhibit about a twofold higher level of luciferase activity than GV oocytes. Results were similar for both *in vivo* and *in vitro* matured oocytes (Fig. 2A). This difference does not depend on transgenic lines, hormonal treatment, culture medium, or presence of follicular cells. It is difficult to explain this increase of

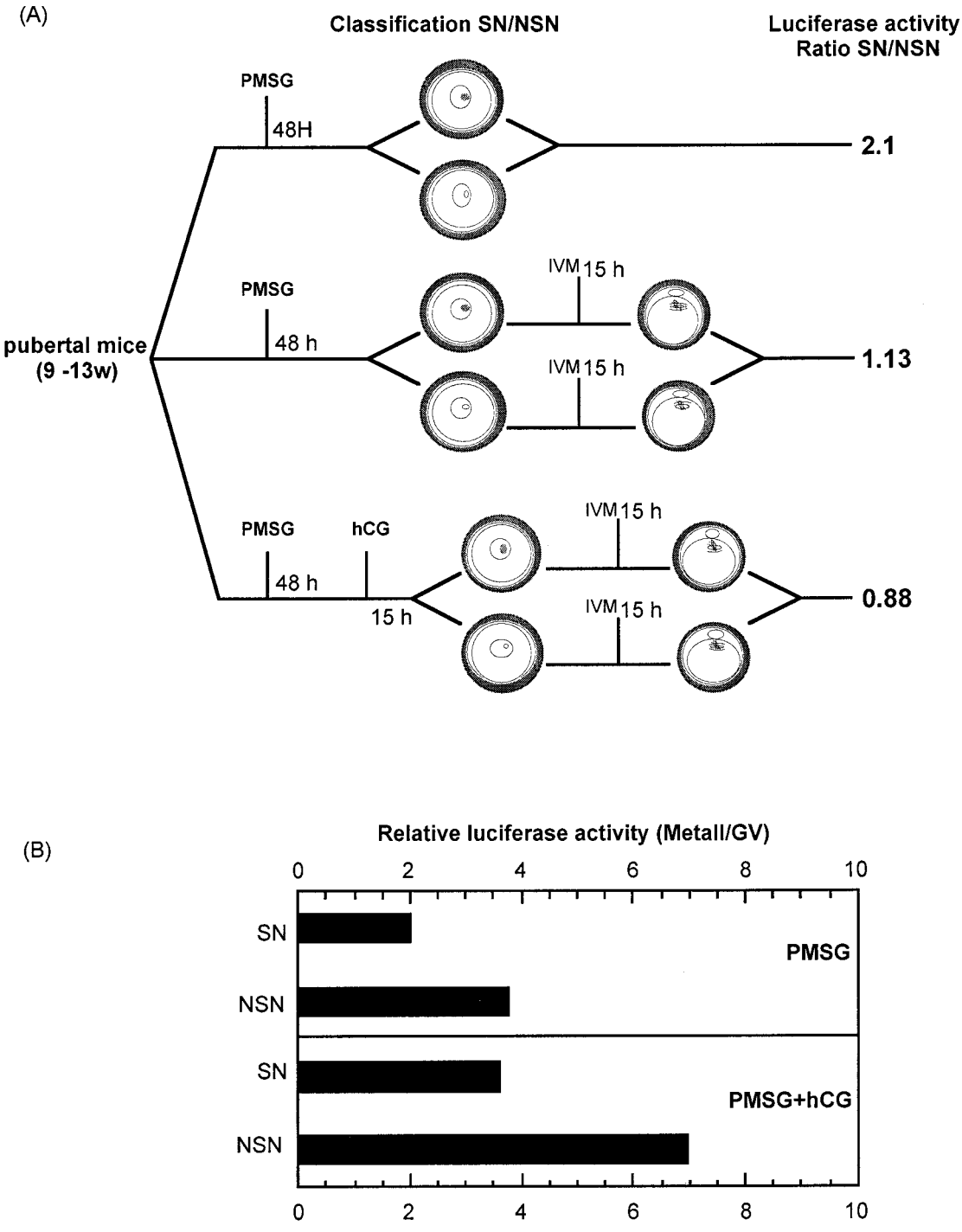


FIG. 3. Comparison of transgene expression in NSN and SN oocytes analyzed at GV stage. (A) Drawing showing the protocol used to obtain the different categories of oocytes assayed for luciferase activity. Mean luciferase activity per oocyte was calculated for SN and NSN oocytes. SN to NSN oocytes ratio of luciferase activity is given for each categories. (B) Effect of maturation on transgene expression in SN and NSN oocytes isolated from the antral compartment 48 h after PMSG injection. Mean luciferase activity calculated for MII oocyte is plotted relative to GV oocyte in both SN and NSN groups.

luciferase activity, which may depend on transcriptional or posttranscriptional changes. It is known that transcription is arrested at the time of GVBD; nevertheless we have verified that transcription is not involved in this change by culturing oocytes in the presence of α-amanitin. This inhibitor of RNA pol II does not inhibit GVBD and has only slight effect on luciferase activity measured in MII oocytes (Fig. 2B). In contrast, inhibition of GVBD by either dbcAMP or IBMX abolished the increase of luciferase activity. Thus, the crucial event is the disappearance of GV, which is accompanied by cytoplasmic dispersion of GV content. We hypothesize that luciferase transcripts could have been stored in GV and then released and further translated after GVBD. Such selective nuclear compartmentalization of transcripts has been described for viral sequences (Ishov *et al.*, 1997). Other posttranscriptional modifications can also be mentioned. Polyadenylation and higher efficiency of translation during maturation have been found for some transcripts (e.g., tPA; Huarte *et al.*, 1987). As in the case of tPA, this effect must be sequence specific as translational activity is globally reduced by 30% during oocyte maturation (Bachvarova and De Leon, 1977, cited in Schultz, 1986). It remains that stability of luciferase itself might be increased during oocyte maturation and in matured oocytes. Thompson *et al.* (1994) have shown that luciferase half-life is about 3 h in cell culture, whereas we have found that it is longer in mouse embryos (about 6 h; Christians *et al.*, 1995). This parameter depends on cell type and can be modified according to physiological status of the cell as it is during oocyte maturation. Unfortunately, we were unable to demonstrate changes in half-life during the course of maturation by use of cycloheximide (Nguyen *et al.*, 1989). When oocytes were classified as SN or NSN and then the same oocytes were cultured to MII, our data showed that the level of luciferase activity was not significantly different in MII oocytes derived from oocytes with the SN morphology and in those derived from NSN oocytes. This equal level of gene expression is reached through an increase of luciferase activity higher for NSN than for SN (see Fig. 3B). Therefore, if our hypothesis is correct a larger amount of untranslated transcripts may be retained in NSN GV oocytes (Fig. 3B). This limited availability of transcripts and corresponding proteins, excluded from the cytoplasm until GVBD, could explain why NSN oocytes have a lower ability to sustain embryonic development beyond the two-cell stage at which major zygotic transcription occurs (Telford *et al*, 1990).

Our work has clearly established that there is a correlation between level of gene expression and SN/NSN patterns of chromatin organization. In order to investigate whether SN/NSN pattern might modulate zygotic genome activation (Christians *et al.*, 1995), we are now planning to analyze HSP70.1Luc transgene expression in preimplantation embryos developed from oocytes with the two different types of chromatin organization. In addition, by using other techniques (i.e., confocal microscopy) it will now be possible to better define the differences in chromatin organization between the two types of oocytes and their intermediate stages (we have demonstrated the existence of these intermediate stages in earlier studies; Zuccotti *et al.*, 1995), to characterize chromosome domains within the nucleus, and to determine the changes in endogenous gene expression when chromatin reorganization occurs during the transition from one type of oocyte to the other or during early development.

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