In contrast, with these experimental conditions, no expression was detected for the *WAP* and α-lactalbumin genes in both LA7 and 106 cells, either in the presence or in the absence of DMSO (data not shown). The induction of domes by lactogenic hormones, together with the presence of the milk proteins *WDNM1* and β-casein in the doming cells, but the lack of the expression of *WAP* and α-lactalbumin genes under these experimental conditions, tend to identify dome-forming cells as components of the lobulo-alveolar structures that develop in the mammary gland at early stage of pregnancy.

Role of Annexin I and *HSP90-β* in Dome Formation. In our previous work, we reported on the proteomic analysis of DMSO-induced LA7 and 106 cells by two-dimensional gel electrophoresis (18). Computer-assisted analysis of the results, performed by using the program MELANIE 3, identified >200 differentially expressed proteins between the two lines. Several of the differentially expressed spots were analyzed by mass spectrometry, using matrix-assisted laser desorption ionization–time-of-flight, and the data obtained were compared to Swiss-Prot and Trembl databases to identify matches to known proteins. We have previously discussed the roles of maspin and tropomyosin-5b, two of the differentially expressed proteins identified (18).

Two additional proteins, annexin I and *HSP90-β*, also showed a high level of expression in DMSO-induced LA7 cells, when compared to 106 cells (Fig. 2). Using the previously described mRNA antisense methodology (16), we determined whether the expression of these genes was essential for dome formation. Inhibition of annexin I or *HSP90-β* protein synthesis was carried out by the addition of mRNA antisense oligonucleotides to LA7 cultures induced by 1.5% DMSO, to see whether dome formation was inhibited. The results, reproduced in Figs. 3*D* and 4*D*, show the complete inhibition of dome formation by either antisense oligonucleotide; in contrast, the corresponding sense (Figs. 3*B* and 4*B*) or scrambled oligonucleotides (Fig. 4 *B* and *C*) were without effect. The expression of these genes is therefore essential for dome formation.

Discussion

In the past, we have studied dome formation in LA7 cells as an example of *in vitro* differentiation, and we considered dome formation as a possible model for studying a specific stage in mammary gland development. This idea was supported by the origin of the cell line LA7 as a clonal derivative from the line RAMA-25 (10), which is obtained from a rat mammary adenocarcinoma and contains mammary stem cells (10–13). Here we present data that confirm that a correlation exists between dome formation *in vitro* and the differentiation processes occurring in the mammary gland at pregnancy. This is based on two new findings: one is that the formation of domes can be induced by the lactogenic hormones HC and PRL, the other is that the formation of domes, induced by either DMSO or lactogenic hormones, in the LA7 cells is accompanied by the production of the milk proteins β-casein and *WDNM1*, which both are specific markers of mammary gland functional differentiation. In addition we show that the DMSO-induced domes express at a high level the annexin I and *HSP90-β* proteins, the expression of which also have been shown to be stage-specific during pregnancy and lactation in other *in vitro* models and in the animal *in vivo* (25, 26). It has been shown that both of these genes are involved in the secretory and functional differentiation of the mammary gland *in vivo* (23, 25, 26); now we demonstrate that their expression in LA7 is required for the formation of domes. We therefore can conclude that the formation of domes corresponds to a specific stage in the lobulo-alveolar development of the mammary gland occurring during pregnancy and lactation.

Distinct steps of cellular differentiation take place during the terminal differentiation of the alveolar epithelial cells; these

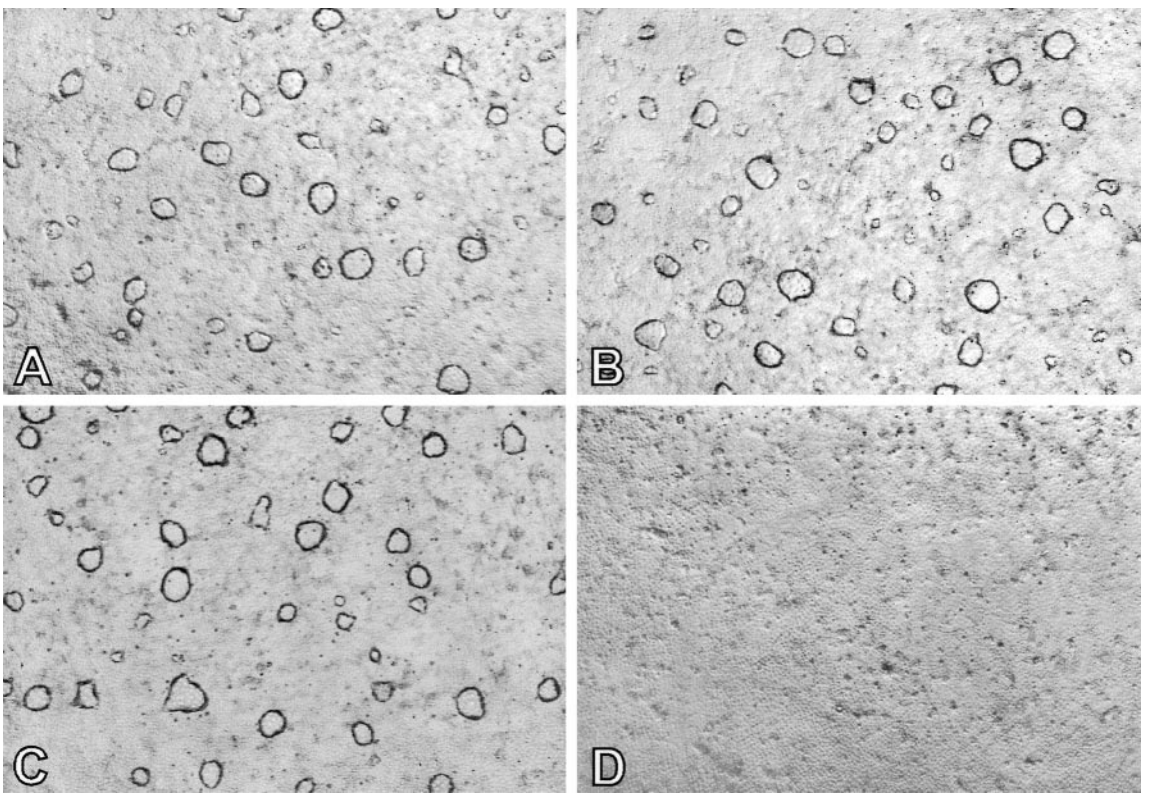

Fig. 3. Antisense anti-annexin I oligonucleotide effect on dome formation in DMSO-induced LA7 cells. (*A*) Cells not treated with oligonucleotides. (*B*) Cells treated with sense oligonucleotides. (*C*) Cells treated with scrambled oligonucleotides. (*D*) Cells treated with antisense anti-annexin I oligonucleotides. (×40.)

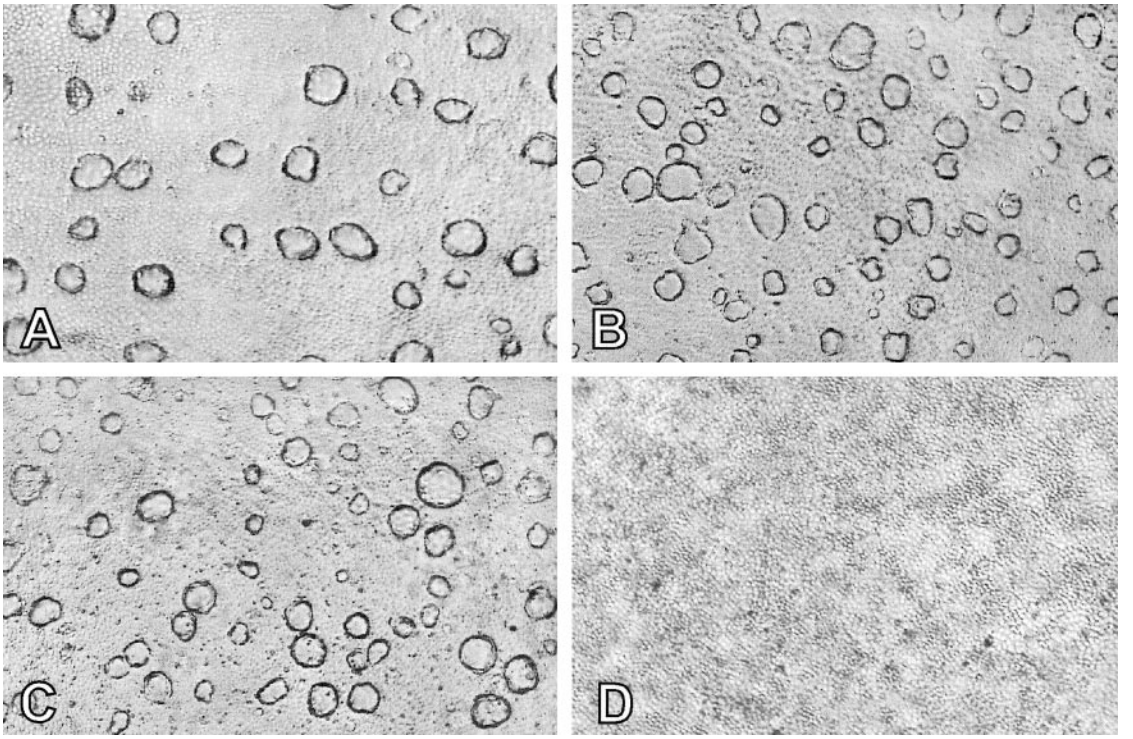

Fig. 4. Antisense anti-HSP90-β oligonucleotide effect on dome formation in DMSO-induced LA7 cells. (*A*) Cells not treated with oligonucleotides. (*B*) Cells treated with sense oligonucleotides. (*C*) Cells treated with scrambled oligonucleotides. (*D*) Cells treated with antisense anti-HSP90-β oligonucleotides. ($\times$40.)

steps are defined by the sequential activation of genes coding for milk proteins (5). The β-casein gene is expressed early during mammary differentiation at pregnancy, closely followed by *WDNM1* (5, 22). Both genes are expressed in the LA7 cells after induction with DMSO. The *WAP* and the α-lactalbumin genes are, in contrast, expressed later in pregnancy, when the terminal differentiation takes place. These two genes were not detected in DMSO-induced LA7 cells, indicating that the developmental stage of DMSO-induced domes in this cell line may correlate to an early stage of lobulo-alveolar development, preceding mammary gland terminal differentiation. Therefore domes in the LA7 cultures correspond to the initial stage of mammary gland development, associated with the formation of tubules and of early alveoli during pregnancy. Consistent with these observations, as reported by Schwarz-Albiez and coworkers (23), the mammary alveolar epithelial cells are the most immunoreactive to annexin I. The LA7 line, therefore, when treated with dome inducers, appears to parallel a distinct stage of the mammary development *in vivo*.

The expression of β-casein and *WDNM1* in the 106 cells may indicate that this line shows an aberrant stage of development, as is possible in a cancer-derived line. In contrast, the LA7 cells has a more physiological behavior. The difference between the two cell lines is supported by the more pronounced tumorigenic growth of 106 cells in nude mice, compared to LA7 cells (I.Z. and R.D., unpublished observations).

The availability of our tissue culture system for studying a stage-specific phase of mammary gland development allows for a simpler method to analyze the expression of individual genes compared to the study carried out in animals because it is directed at a single cell type that differentiates *in vitro*. Moreover, the roles of the expressed genes can be determined by the simple approach of the antisense RNA technology. The same system also should be suitable for determining the signals that cause the undifferentiated LA7 cells to differentiate in the lobulo-alveolar direction.

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