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Cell adhesion alters gene transcription in chicken embryo brain cells and mouse embryonal carcinoma cells

(cell adhesion molecule/gene expression/subtractive hybridization)

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ABSTRACT **To determine whether changes in gene expression occur in embryonic cells as a consequence of changes in cellular aggregation, chicken embryo brain (CEB) cells isolated from 8-day embryos were allowed to aggregate or prevented from aggregating by treatment with anti-neural cell adhesion molecule (N-CAM) Fab' fragments. A subtractive hybridization cloning strategy was employed to identify genes that might show different levels of expression in the two populations of cells. In addition, the transcription rates of a number of genes specifying CAMs and transcription factors were directly estimated by using nuclear run-off transcription assays. The transcription rates of several genes, including those encoding N-CAM, Ng-CAM, α-N-catenin, HoxA4 (Hox1.4), a fatty acid-binding protein, and a subunit of the mitochondrially encoded cytochrome-*c* oxidase enzyme decreased upon CEB cell aggregation. The transcription rates of several previously unidentified genes either increased or decreased upon aggregation, while the transcription of other genes remained unchanged. The transcription rate of the N-CAM gene was 3.3-fold higher in dissociated than in aggregated CEB cells. This rate of transcription also increased when the brain tissue was dissociated into single cells and the increased rate was maintained by keeping the cells dissociated in the presence of Fab' fragments of antibodies to N-CAM. Decreased transcription rates of the N-CAM gene were also observed upon aggregation of P19 cells, a mouse embryonal carcinoma cell line. Primary chicken embryo liver cells, which aggregate primarily by calcium-dependent adhesion mechanisms, did not show changes in the N-CAM gene or in the other genes whose transcription rates changed in CEB cells and P19 cells. These observations suggest that the types of genes regulated by cell aggregation include those for CAMs themselves as well as for transcription factors that may control the expression of CAMs and other molecules significant for morphogenesis.**

Cell adhesion molecules (CAMs) and substrate adhesion molecules (SAMs) govern the interactions of cells both with other cells and with extracellular matrix molecules during development (1, 2). Various combinations of these molecules are expressed in place-dependent patterns during the development of a given species and lead to characteristic morphologies. Two questions arise in connection with this place-dependent expression. (*i*) What genes are responsible for the regulation of CAM and SAM expression? (*ii*) How do changes in cell adhesion mediated by CAMs and SAMs in turn regulate the transcription of developmentally significant genes, including those specifying CAMs and SAMs themselves? Recent evidence bearing upon the first question suggests that the developmental expression of some CAMs and SAMs is regulated in part by the products of homeobox-containing genes (3–5) or pax genes (B. D. Holst, R. J. Goomer, F. S. Jones, and G.M.E., unpublished work), which are known to control pattern formation in particular regions of the embryo (6).

In the present study, we address the second question, focusing on whether CAM-mediated cell aggregation *in vitro* can lead to variation in gene expression. Using well-established assays (7, 8) for short-term calcium-independent cell aggregation, we found that chicken embryo brain (CEB) cells and P19 mouse embryonal carcinoma cells differ in their expression of specific genes depending on whether they are in a dissociated or an aggregated state. Genes identified by a subtractive hybridization cloning strategy (9, 10) that showed a higher rate of transcription in dissociated cells included those encoding N-CAM, α-N-catenin, a subunit of cytochrome-*c* oxidase, and a fatty acid-binding protein. By directly measuring the rate of transcription (11) of genes for various CAMs and homeobox-containing transcription factors, additional genes were identified that had higher transcription rates in dissociated cells, including those encoding Ng-CAM, HoxA4 (Hox1.4), and Evx-1. Furthermore, a number of novel genes whose transcription rates differed between aggregated and dissociated cells were identified by the combined use of the subtractive and run-off methodologies.* These approaches provide a basis for the identification and characterization of other genes that are modulated in various tissues in response to CAM-mediated cell interaction.

MATERIALS AND METHODS

Aggregation Assays. CEB cells were prepared from 8-day chicken embryo brains by limited trypsinization in the presence of EDTA (8, 12). Mouse embryonal carcinoma (P19) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 2.5% fetal bovine serum, 7.5% calf serum, glutamine, and penicillin/streptomycin. Aggregation of CEB and P19 cells was performed in suspension MEM (s-MEM)/20 mM Hepes, pH 7.4/1 mM $CaCl_2$ at 37°C as described (12), with minor modifications for the P19 cells. P19 cell aggregation was carried out in 6-well rather than 24-well multiplates and the cells were shaken at 50 rpm rather than 100 rpm. CEB cells were kept in the dissociated state by including anti-chicken N-CAM Fab' fragments (0.1 mg/ml) in the aggregation reaction. P19 cells were kept dissociated by plating onto tissue culture plates. The cells on plastic were only weakly attached and did not flatten; they were easily removed from the plastic by pipetting at the end of the experiment.

Library Construction. Poly(A)$^+$ RNA was isolated from aggregated or dissociated 8-day CEB cells. Directional cDNA libraries were constructed in the Uni-ZAP XR vector

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Abbreviations: CAM, cell adhesion molecule; CEB, chicken embryo brain.

*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. X77852 and X77853).

(Stratagene) so that the T3 RNA polymerase promoter was located at the 5′ end of the cloned cDNA. The first-strand cDNA was synthesized by using an oligo(dT) *Xho* I linker. After second-strand cDNA synthesis, *Eco*RI adaptors were added, the cDNA was digested with the restriction enzymes *Eco*RI and *Xho* I and cloned into λZAP II (Stratagene). The libraries were packaged with Gigapack II extracts (Stratagene). Phage λ DNA was prepared from each of the libraries and was cut with *Xho* I to obtain templates for RNA transcription by T3 RNA polymerase.

Subtractive Hybridization. RNA transcripts of the clones generated by T3 RNA polymerase from each of the libraries were reverse transcribed to make first-strand cDNA by using the oligo(dT) *Xho* I linker. Biotin-labeled RNA was also produced from each of the libraries (10). Two micrograms of first-strand cDNA from each of the libraries from aggregated (A) or dissociated (D) cells was hybridized with 30 μg of biotin-labeled RNA from the other library (either D or A) in 4 μl containing 50 mM Hepes (pH 7.6), 0.2% SDS, 2 mM EDTA, and 500 mM NaCl. The hybridization mixtures were overlaid with mineral oil, heated to 95°C for 2 min, and incubated at 65°C for 2 days. The mixtures were recovered and diluted to 100 μl with the same hybridization buffer lacking SDS, 5 μg of streptavidin was added, and the mixture was phenol/chloroform extracted. The organic phase was washed to recover all of the DNA and the aqueous phase was extracted twice more with fresh streptavidin. Nucleic acids were precipitated by addition of 10 μg of biotin-labeled RNA to ensure complete recovery of the remaining cDNA. A further 20 μg of biotin-RNA was added and the hybridization was repeated. After two and six rounds of hybridization, the cDNA remaining was amplified by PCR using the oligo(dT) *Xho* I linker and the pBluescript SK primer (Stratagene) for 25 rounds, each consisting of denaturation at 94°C for 1 min, annealing at 40°C for 1 min, and extension at 72°C for 1 min. The resulting products were digested with *Eco*RI and *Xho* I and cloned into λZAP II. These subtracted libraries were plated and duplicate filters were hybridized with labeled cDNA probes made from aggregated and dissociated CEB cells. Filters were hybridized and washed at high stringency (15 mM NaCl/1.5 mM sodium citrate, pH 7.0/0.1% SDS at 65°C). Plaques that hybridized more strongly with one probe were selected for further analysis.

Nuclear Run-Off Assays. Nuclei were isolated (13), suspended in 200-μl aliquots containing 2×10^7 nuclei, and stored at −70°C until use. Nuclear run-off probes consisting of labeled nascent RNA transcripts from aggregated and dissociated cells were produced by incubating thawed nuclei with [α-^{32}P]UTP and unlabeled ATP, CTP, and GTP (13). RNA probes were extracted with RNA-Now (BioGentex, Seabrook, TX) and precipitated with 2-propanol (14). Samples containing equivalent incorporated counts from each of the probes were hybridized to duplicate slot blots in Rapid-hyb buffer (Amersham) at 65°C overnight. After hybridization, the blots were washed four times for 15 min at high stringency. The blots were exposed for various times with a PhosphorImager (Molecular Dynamics); images were obtained and analyzed with IMAGEQUANT software (Molecular Dynamics). The hybridization signals represent the pixel values above background that were obtained from the images; these were normalized against values obtained for tubulin, actin, and U6 small nuclear RNA hybridizations as described in *Results*.

Labeled cDNA. T3 polymerase-transcribed RNA from libraries A and D was reverse transcribed to yield first-strand cDNA by using oligo(dT) as a primer. Twenty nanograms of first strand cDNA was labeled by random oligonucleotide priming (Boehringer Mannheim) and hybridized to cDNA clones immobilized onto Hybond-N$^+$ (Amersham). All hybridizations and washes were performed as described above.

DNA Slot Blots. cDNA clones (10 μg) were denatured by boiling, chilled on ice, and slot-blotted onto Hybond-N$^+$. DNA was fixed by placing the membrane on Whatman 3MM paper soaked with 0.4 M NaOH for 10 min, followed by a rinse with 300 mM NaCl/30 mM sodium citrate, pH 7.0. Filters were prehybridized in Rapid-hyb buffer prior to hybridization.

Sequence Analysis. For sequence analysis, pBluescript plasmids were obtained by *in vivo* excision from plug stocks of λ clones by using R408(f1) helper phage (Stratagene). Plasmid DNA was prepared and sequenced by the dideoxynucleotide chain-termination method (15) using Sequenase (United States Biochemical). Searches for relationships with known sequences were performed with the FASTA program (Genetics Computer Group, Version 7.2-UNIX) to scan the GenBank (release 80), EMBL (release 35), and Swiss-Prot (release 26) databases.

RESULTS

Two approaches were used to identify changes in gene expression following cell aggregation. The first was to prepare subtracted cDNA libraries from dissociated or aggregated CEB cells, with the aim of isolating sequences preferentially expressed in each population. The second approach was to examine differences between aggregated and dissociated cells in the rates of transcription of particular genes for which probes were available. This was accomplished by hybridizing radioactively labeled probes prepared from aggregated or dissociated cells to cDNA sequences for known genes. Two types of radioactive probe were used: cDNA probes to measure mRNA levels and nuclear run-off probes to measure rates of transcription. In both approaches, we chose to examine cells known to aggregate by means of N-CAM and used either specific anti-N-CAM Fab′ fragments or cells plated in a dissociated state to maintain disaggregation.

Identification of cDNA Clones of Genes Differentially Transcribed in Aggregated or Dissociated CEB Cells. The methodology by which cDNA libraries were prepared from dissociated (D) or aggregated (A) CEB cells is illustrated in Fig. 1*a*. Sequences in library A were subtracted from library D to enrich for sequences present in dissociated cells (library D-A, Fig. 1*a*). Similarly, sequences in library D were subtracted from library A to enrich for sequences present in aggregated cells (library A-D). The number of rounds of subtraction necessary to remove common sequences was monitored by a test protocol in which radiolabeled first-strand cDNA and biotin-labeled RNA were both transcribed from cDNA library A (library A-A). The amount of radioactivity remaining in the first-strand cDNA was followed for six rounds of subtractive hybridization (Fig. 1*b*). Subtracted cDNA libraries A-D and D-A were made after two and six rounds of subtraction. After four rounds of subtractive hybridization, ≈90% of the sequences were removed; the remaining 10% could not be removed even after an additional two rounds of subtractive hybridization. This background DNA consisted of a single sequence that did not encode any known protein but showed marginal DNA similarities with a number of viral genome sequences. This clone comprised 30% of libraries D-A and A-D prepared after six rounds of subtraction.

The cDNA clones that were differentially enriched in the libraries were selected by probing with first-strand cDNA probes prepared from aggregated or disaggregated cells. Enriched clones were found in both the D-A and A-D libraries. One of the clones found to be enriched in the D-A library (after two rounds of subtraction) encoded N-CAM itself. This observation suggested that the transcription of the N-CAM gene was greater in dissociated than in aggregated CEB cells.

Transcriptional Regulation of the N-CAM Gene. Transcription was measured by nuclear run-off analysis. An equivalent

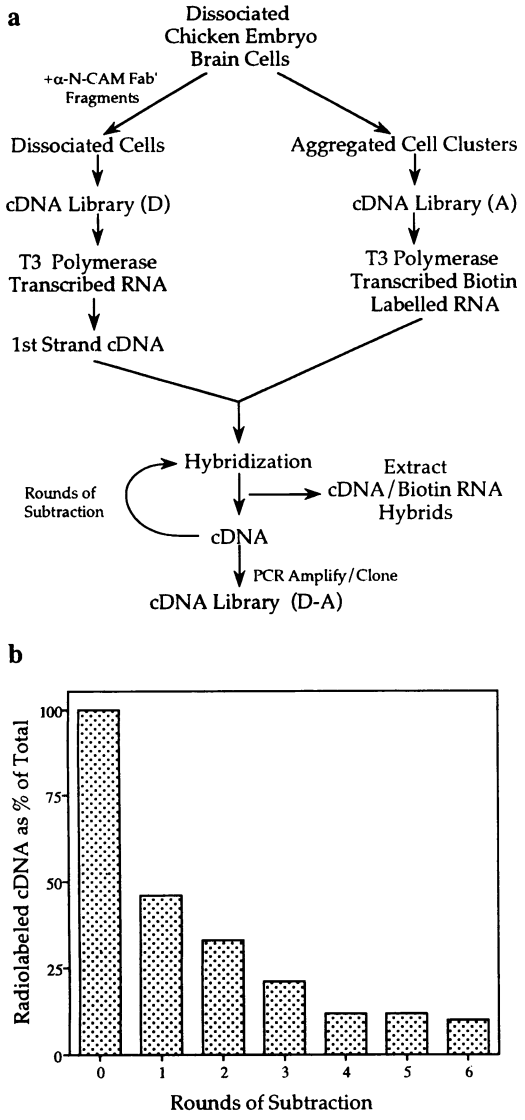

FIG. 1. Subtractive hybridization. (*a*) Methodology used to isolate cDNA clones that are preferentially expressed in aggregated or dissociated CEB cells (see text). (*b*) Quantitation of the efficiency of subtraction. The extent of subtraction of common cDNA clones was monitored by using radioactive cDNA from library A and biotin-labeled RNA from the same library. The amount of radiolabeled cDNA remaining was followed for six rounds of subtractive hybridization.

number of incorporated counts of run-off transcription probes from aggregated or dissociated CEB cells resulted in equivalent signals for many genes, including those for actin, tubulin, and U6 small nuclear RNA. The transcription levels of these three genes were therefore chosen to normalize the hybridization results.

We found that the normalized N-CAM transcription rate was 3.3-fold higher in dissociated CEB cells than in aggregated CEB cells. Transcription of the N-CAM gene was then measured as a function of time after reaggregation (Fig. 2). Nuclear run-off transcription probes were prepared from intact embryonic day-8 CEB tissue, from single cells dissociated from day-8 CEB tissue (ref. 8; see *Materials and Methods*), and from these cells after they were allowed to aggregate for various times or after they were prevented from aggregating by the presence of Fab' fragments of antibodies to N-CAM (Fig. 2). The rate of transcription of the N-CAM gene increased ≈3-fold in dissociated cells relative to the intact brain tissue. When the cells were allowed to aggregate,

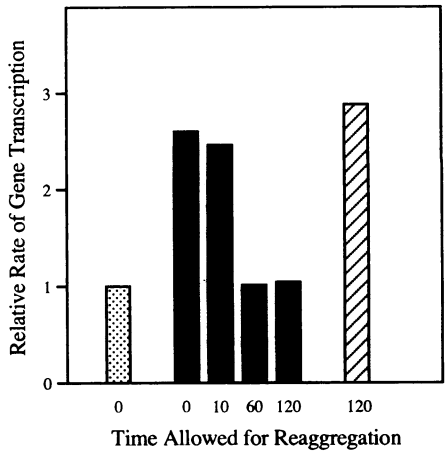

FIG. 2. Time course of N-CAM gene transcription in aggregated or dissociated CEB cells. Nuclear run-off transcription probes were made from intact 8-day CEB tissue (0, stippled bar), from dissociated cells after aggregating for 0, 10, 60, or 120 min (black bars), or after 120 min in the presence of anti-N-CAM Fab' fragments (hatched bar). Values on the ordinate reflect the rate of transcription relative to that seen in the intact brain tissue (stippled bar).

the rate of transcription decreased within 60 min to the level seen in intact brain tissue. Cells prevented from aggregating by the presence of anti-N-CAM Fab' fragments showed a rate of transcription similar to that seen in single cells.

Transcriptional Responses of P19 Cells. To determine whether similar transcriptional changes occurred in another cell type, we used P19 cells, a mouse embryonal carcinoma cell line that can be induced to differentiate into a number of different cell types, including neurons and astrocytes (16). These cells express N-CAM and aggregate in a calcium-independent manner. Nuclear run-off transcription analysis comparing aggregated with dissociated cells gave results similar to those obtained with CEB cells: in dissociated cells, the transcription of the mouse N-CAM gene was ≈3-fold higher than in aggregated cells.

Identification of Other Genes That Respond to Cell Aggregation. Several other genes in addition to the N-CAM gene were found to change their expression as a function of cell aggregation (Table 1). In addition to the genes identified in the subtractive libraries, a screen of other CAM genes and homeobox-containing genes available in the laboratory revealed those specifying Ng-CAM and HoxA4 as genes whose expression was greater in dissociated than in aggregated CEB cells (Table 1, part a). Moreover, a number of additional cDNA clones were identified from the D-A library (prepared after two rounds of subtraction) whose mRNA levels and transcription rates decreased in aggregated cells (Table 1, part a). The partial sequences of four of these clones are shown in Fig. 3. Three of the clones encoded known proteins. One was 100% identical in a 71-bp overlap to chicken α-N-catenin (17) (Fig. 3*a*), a protein associated with the cytoplasmic domain of CAMs of the cadherin family (17, 18). Two other genes, apparently not directly related to cell adhesion or transcriptional regulation, were 99% identical in a 99-bp overlap to a subunit of chicken mitochondrial cytochrome-*c* oxidase (19) (Fig. 3*b*) and 100% identical in an 89-bp overlap to the chicken fatty acid-binding protein (20) (Fig. 3*c*). The fourth clone (Fig. 3*d*) encoded an open reading frame of 40 amino acids (D-A no. 26), but this was not homologous to any known protein or DNA sequences.

cDNA clones were also isolated from the A-D (Table 1, part b) library that showed differential expression but whose sequences did not correspond to those of any known proteins. The sequence of one of these clones (A-D no. 1) is

Table 1. Differences in gene expression between aggregated and dissociated cells

cDNA clone	CEB cells: cDNA analysis	CEB cells: Nuclear run-off	P19 cells: Nuclear run-off
(a) Greater expression in dissociated cells			
N-CAM*	2.2	3.3	3.0
Ng-CAM*	1.3	3.1	2.9
α-N-Catenin (D-A no. 27)	1.8	2.0	—
HoxA4*	1.4	2.3	—
Cytochrome-*c* oxidase (D-A no. 13)	2.3	4.9	2.4
Fatty acid-binding protein (D-A no. 29)	1.8	4.0	—
Evx-1*	—	—	2.4
D-A no. 26	2.0	1.8	
D-A no. 28	2.1	2.2	
(b) Greater expression in aggregated CEB cells			
A-D no. 1	—	2.3	
A-D no. 143	3.5	—	
A-D no. 25	—	2.0	
(c) No difference between aggregated and dissociated CEB cells			
HoxA3*	—	1.0	
D-A no. 6	—	1.2	
D-A no. 7	1.1	0.7	
D-A no. 34	1.0	1.1	
A-D no. 9	1.1	1.2	
A-D no. 10	—	1.2	

CEB and P19 cells were aggregated or dissociated as described in *Materials and Methods*. cDNA and nuclear run-off analyses were quantitated and normalized as described. Changes in gene expression were measured as the hybridization signal obtained with nuclear run-off probes from dissociated relative to aggregated cells (*a* and *c*) or from aggregated cells relative to dissociated cells (*b*). The nuclear run-off values are the average of at least two independent experiments.
*Changes in the transcription of these genes were directly probed for using previously isolated cDNA clones. The N-CAM clone used in the P19 cell experiment and the Evx-1 cDNA clone were from mouse; all the other clones were from chicken. N-CAM was identified in the subtractive libraries (D-A no. 4), but in the analysis of CEB cells presented here, a previously isolated cDNA clone for chicken N-CAM was used.

presented in Fig. 3*e*. Several hundred clones were also analyzed whose expression was the same in aggregated or dissociated cells or whose expression was undetectable by the methods used. Six of these are listed in Table 1, part c.

To test whether the genes listed in Table 1, part a, were regulated in the same way in P19 cells, nuclear run-off probes from these mouse cells that had been aggregated or kept dissociated were hybridized to chicken and mouse cDNA clones. A mouse N-CAM clone, as well as chicken clones encoding Ng-CAM and a subunit of cytochrome-*c* oxidase, revealed transcription that was increased in dissociated P19 cells. In addition, transcription of the gene encoding Evx-1, another homeobox-containing transcription factor, was increased in dissociated relative to aggregated P19 cells; no Evx-1 hybridization could be detected with run-off transcription probes made from CEB cell nuclei. The other chicken genes (Table 1, part a) whose transcription changed in CEB cells were not detectable with the mouse run-off probes.

The transcription rates in dissociated CEB cells of a number of the genes shown in Table 1 were compared with those found in cells from intact whole brain tissue. The genes whose transcription was greater in dissociated than in aggregated cells increased their rate of transcription when brain tissue was dissociated into single cells. Conversely, clone A-D no. 25, which had a greater rate of transcription in aggregated cells, also had a greater rate of transcription in brain tissue than in

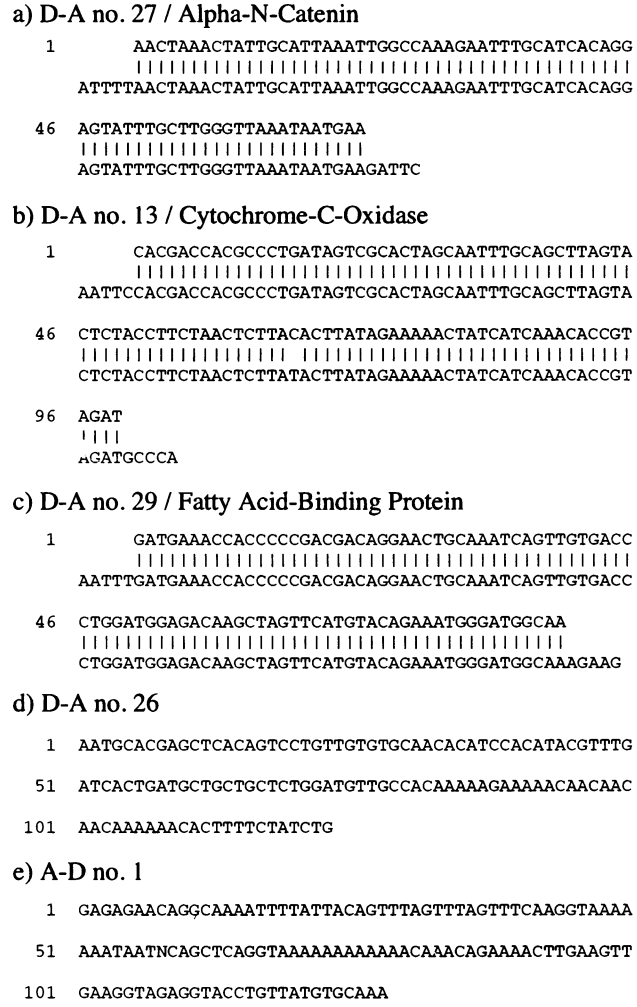

FIG. 3. cDNA sequences of five clones isolated from subtracted libraries. (*a–d*) Partial sequences of four clones isolated from the D-A library (see Table 1); three of these (*a–c*) encode known proteins based on sequence comparisons. Upper sequences in *a–c* are of the D-A subtracted clones nos. 27, 13, and 29 and are compared (vertical lines) with lower sequences obtained from the database. *a* is α-N-catenin, *b* is a subunit of mitochondrial cytochrome-*c* oxidase, and *c* is a fatty acid-binding protein. *d* contains a single open reading frame of 40 amino acids with no apparent homology to known sequences. (*e*) A clone isolated from the A-D library that also showed no apparent homologies with known sequences.

dissociated cells. The results suggest that transcriptional changes occur initially upon tissue dissociation into single cells but can be reversed in response to reaggregation.

To compare the responses of cells whose adhesion is mediated by calcium-dependent CAMs for the genes examined here, we investigated whether a nonneuronal cell type would respond to cell aggregation/dissociation in the same fashion as CEB and P19 cells. Embryonic day-8 liver cells were aggregated in the presence of calcium and nuclear run-off transcription assays were performed. No changes were observed between aggregated and dissociated liver cells in the transcription rate of N-CAM or of any of the other genes encoding known proteins listed in Table 1, part a.

DISCUSSION

To identify genes whose expression changes as a consequence of cell aggregation, we have used a subtractive hybridization strategy to prepare cDNA libraries enriched for sequences that are preferentially expressed in either aggregated or dissociated CEBs. We identified examples of genes

whose rate of transcription decreased, increased, or did not change in response to cell aggregation. Several of the identified genes encoded known proteins, including N-CAM, the CAM whose homophilic interaction was perturbed in these experiments to maintain the cells in a dissociated state.

The transcription of several genes was found to decrease as a result of CAM-mediated cell aggregation in CEB cells and P19 cells, both of which can aggregate by means of N-CAM. The genes encoding N-CAM, Ng-CAM, α-N-catenin, HoxA4, a fatty acid-binding protein, and a subunit of cytochrome-*c* oxidase all increased their expression in dissociated relative to aggregated CEB cells. The observed changes in gene expression appear to be directly related to cell aggregation and are not simply the result of the procedures used in the cell dissociation or of the presence of Fab' fragments of anti-N-CAM antibodies. For example, when CEB cells were dissociated, transcription of the N-CAM gene and other genes increased relative to the levels seen in intact tissue (Fig. 2). Transcription remained at this elevated level when the cells were kept dissociated by Fab' fragments of antibodies to N-CAM. When the cells were allowed to aggregate, the rate of transcription decreased toward the level seen in intact CEB tissue. In contrast, chicken embryo liver cells, which aggregate primarily by a calcium-dependent mechanism that does not involve N-CAM, did not show changes in the genes for N-CAM or the other genes whose transcription rates were changed in CEB cells; whether other genes are differentially transcribed after dissociation and reaggregation in this cell type remains to be determined. Moreover, P19 cells, a mouse embryonal carcinoma cell line, responded in a manner similar to CEB cells even though the method used to keep the cells dissociated did not involve antibodies. All of these results suggest that the observed transcriptional changes occur in response to cell aggregation. It is not known whether CAM–CAM interactions that directly alter signaling (1, 21–23) can affect gene expression or whether signals from other cell surface molecules may be facilitated by such interactions.

The observation that genes encoding two neural CAMs, N-CAM and Ng-CAM, and a protein that associates with cadherins, α-N-catenin, had increased rates of transcription in dissociated relative to aggregated CEB and P19 cells suggests that a feedback mechanism mediated by CAM binding may be operating in the cells. CAM-mediated cell aggregation resulted in the down-regulation of several genes involved in the cell adhesion process; conversely, dissociation of embryonic tissue into cells resulted in the upregulation of these genes. A suggestion that such feedback mechanisms may operate *in vivo* is provided by the observation that transgenic mice expressing chicken L-CAM under the control of the rat insulin II promoter appear to downregulate the expression of the endogenous mouse L-CAM gene in the β cells of the pancreas (24). A more detailed analysis of CAM promoters (refs. 4 and 5; B. D. Holst, R. J. Goomer, F. S. Jones, and G.M.E., unpublished work) may allow us to identify the sequences and transcriptional mechanisms underlying these responses to cell aggregation.

Of the other genes identified, *HoxA4* and *Evx-1* are of particular interest because they encode homeodomain transcription factors that are expressed in a place-dependent manner in the developing mouse nervous system (25, 26). Evx-1 has been shown to regulate the transcription of the cytotactin gene *in vitro* (3) and may affect the expression of other adhesion-related genes. Moreover, several *hox* genes have been shown in *in vitro* studies to regulate the expression of the N-CAM gene (4, 5). Identifying the targets of HoxA4 and Evx-1 may therefore be of particular interest in understanding regulatory responses to cell aggregation.

Extension of the current observations to other cellular systems may help to clarify how interactions via CAMs whose genes are targets of homeodomain transcription factors can affect expression of various genes, including those encoding homeodomain transcription factors whose expression may lead to changes in morphogenesis or histodifferentiation. This directs attention to the analysis of the targets of such transcription factors and to the exploration of other novel genes whose expression is changed after cell aggregation. Further characterization of the sequences present in the subtractive libraries prepared in this study, for example, should provide a better understanding of the transcriptional changes that occur as a consequence of cell aggregation. Such studies may shed light on how different states of cell aggregation and interaction via combinations of different CAMs (1, 21) can affect the expression of morphoregulatory genes during development.

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