Induction of Proliferation or Hypertrophy of Chondrocytes in Serum-free Culture: The Role of Insulin-Like Growth Factor-I, Insulin, or Thyroxine

Kathrin Böhme, Marianne Conscience-Egli, Thomas Tschan, Kaspar H. Winterhalter, and Peter Bruckner

Laboratorium für Biochemie, Eidgenössische Technische Hochschule, CH-8092 Zürich, Switzerland

Abstract. In bone forming cartilage in vivo, cells undergo terminal differentiation, whereas most of the cells in normal articular cartilage do not. Chondrocyte hypertrophy can be induced also in vitro by diffusible signals. We have identified growth factors or hormones acting individually on 17-d chick embryo sternal chondrocytes cultured in agarose gels under strictly serum-free conditions. Insulin-like growth factor I or insulin triggered the first steps of chondrocyte maturation, i.e., cell proliferation and increased matrix deposition while the chondrocytic phenotype was maintained. However, cells did not progress to the hypertrophic stage. Proliferation and stimulated collagen production was preceded by a lag period, indicating that synthesis of other components was required before cells became responsive to insulin-like growth factor I or insulin. Very small amounts of FBS exerted effects similar to those of insulin-like growth factor I or insulin. However, FBS could act directly and elicited hypertrophy when constituting >1% of the culture media. Basic FGF has been claimed to be the most potent chondrocyte mitogen, but had negligible effects under serum-free conditions. The same is true for PDGF, a major serum-mitogen. Under the direction of thyroxine, cells did not proliferate but became typical hypertrophic chondrocytes, extensively synthesizing collagen X and alkaline phosphatase.

CARTILAGE cells in situ modulate their phenotype in response to environmental stimuli. Pluripotent mesenchymal cells differentiate into resting chondrocytes characterized by a moderate rate of synthesis of aggregating proteoglycans and collagens II, IX, and XI. Most of the cells maintain this phenotype in joints where cartilage is permanent. During endochondral ossification in development, growth, and repair of bones, however, resting chondrocytes differentiate further. Initially, they proliferate and, later, they become hypertrophic in that they drastically increase both their volume and metabolic activity. Hypertrophic cells deposit large quantities of extracellular matrix containing cartilage-specific proteoglycans and collagens including collagen X, a marker for this stage of differentiation (Schmid and Linsenmayer, 1985). In contrast to resting and proliferative cartilage cells, hypertrophic cells also produce alkaline phosphatase.

The phenotypic flexibility of chondrocytes is also apparent in vitro. In monolayer culture, the cells can gradually lose the cartilage phenotype and begin to resemble mesenchymal cells (Holtzer et al., 1960) and they produce macromolecules not normally found in cartilage (Mayne et al., 1976; von der Mark et al., 1977). By contrast, in suspension culture stabilized by semisolid gels (Horwitz and Dorfman, 1970), chondrocytes remain differentiated and cells modulated in monolayer culture reexpress the cartilage phenotype

in agarose (Benya and Shaffer, 1982) or collagen gels (Gibson et al., 1982). These observations led to the conclusion that cell–matrix interactions were responsible, at least in part, for the program of gene expression in cartilage. An attractive possibility for the mechanism of matrix-induced effects on cells consists of indirect effects on signaling molecules (Nathan and Sporn, 1991). In this concept, matrix components may regulate cellular activities by binding growth factors or hormones, thereby concentrating or inactivating them. Degradation of stationary components binding diffusible signals could well lead to an increased response by the tissue.

Metabolic regulation in cartilage by growth factors has been extensively studied in vivo or in organ or cell culture (for reviews see Canalis et al., 1989; Humbel, 1990; Froesch et al., 1985; Gospodarowicz, 1990; Massagué, 1990; Heldin and Westermark, 1989; Ross et al., 1986). However, effects elicited in vivo are frequently difficult to attribute to specific signaling molecules because systemic components can interfere. Likewise, the interpretation of tissue culture experiments is complicated because chondrocytes are frequently kept in monolayer culture or in media containing serum, or both. In addition, cultures have often been designated as serum-free, when the media contained very low amounts of serum or when serum was removed only prior to growth factor-treatment. For chondrocytes, this is partic-
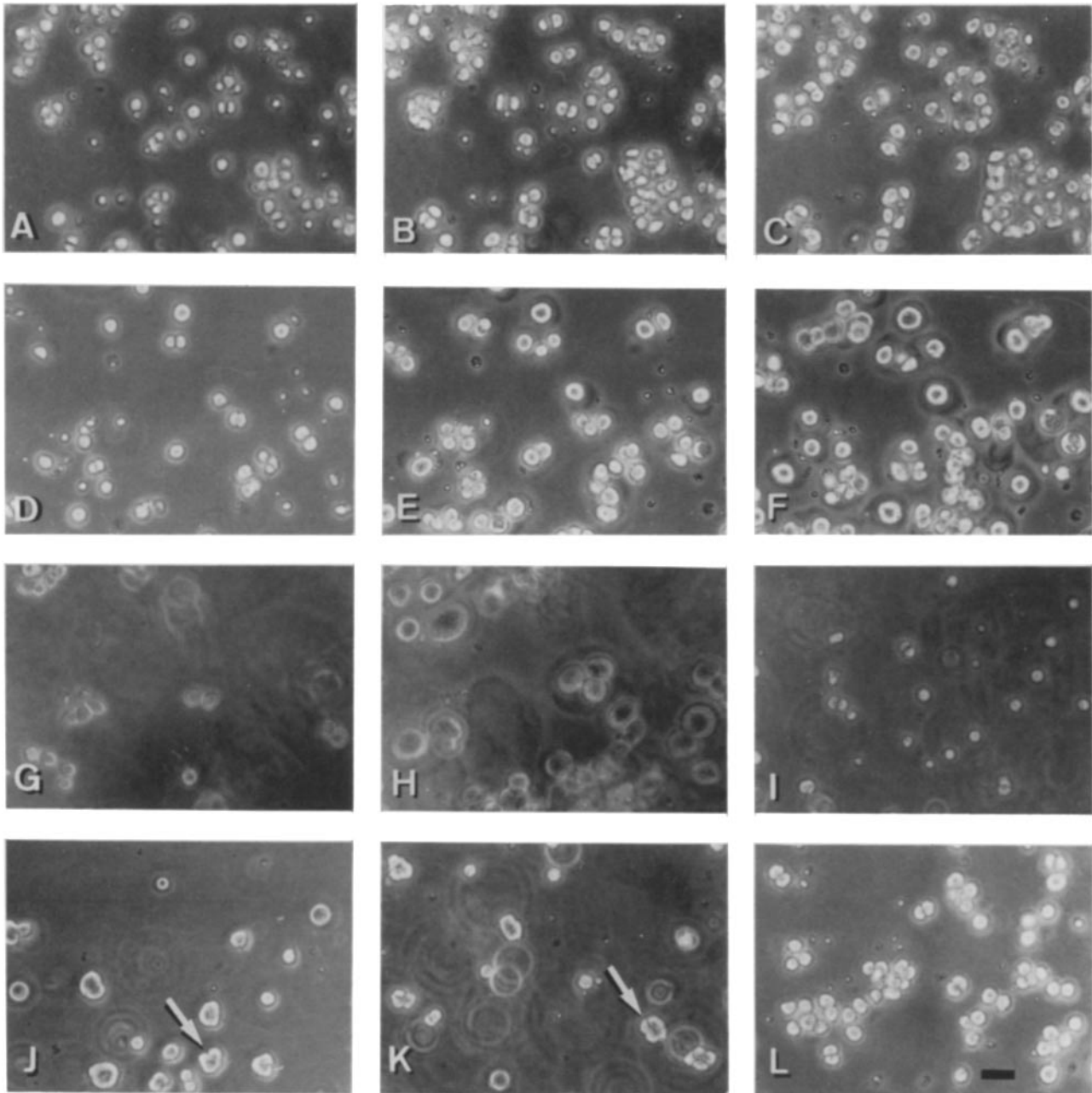
Figure 1. Development of chick embryo sternal chondrocytes in vitro. Phase-contrast light micrographs of cells treated with FBS (*A–C*, 0.1%; *D–F*, 1%; *G* and *H*, 10%), 10 ng/ml of basic FGF (*J*), 50 ng/ml PDGF (*K*), or 100 ng/ml of IGF-I (*L*). Control cells (*I*) were grown in DME containing 1 mM cysteine, only. The culture periods were: 3 d (*A, D,* and *G*), 7 d (*B, E, H,* and *L*), and 14 d (*C, F, I, J,* and *K*). The same micrographic field is shown for each culture condition in *A–H*. Note that cells treated with either basic FGF or PDGF are irregularly shaped and form small clusters (*arrows* in *J* and *K*). Bar, 50 μm.

ularly inappropriate because FBS by itself acts as a strong mitogen and can induce the entire cascade of terminal differentiation (Bruckner et al., 1989).

Primary chondrocytes from 17-d-old chick embryo sterna maintain the resting phenotype in serum-free gel-suspension culture. They are viable in DME containing pyruvate or cysteine and, hence, chemically undefined culture media can be avoided (Tschan et al., 1990). These culture conditions offer a unique opportunity to study the influence of individual signaling molecules on both proliferation and induction of hypertrophy in chondrocytes. Here, we provide such infor-

mation on insulin-like growth factor type I (IGF-I)[1], insulin, basic FGF, PDGF, and thyroxine.

Materials and Methods

Agarose Cultures

17-d-old chick embryo sternal chondrocytes were grown in serum-free agarose suspension culture in 35-mm Petri dishes at a density of 2×10^6

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1. *Abbreviation used in this paper*: IGF-I, insulin-like growth factor type I.

Table I. Chondrocyte Proliferation in Response to FBS

	FBS	Increase in cell numbers at day			
		3	7	10	14
	%				
Experiment 1	0	0.85	0.87	1.09	1.03
	0.1	0.83	1.04	1.16	1.20
	1	0.90	1.27	1.62	2.46
	10	1.61	1.70	3.30	2.38
Experiment 2	0	1.19	1.15	1.10	1.15
	0.1	1.05	1.71	1.82	1.97
	1	1.15	1.95	4.23	5.43

The numbers represent the cell numbers observed in a particular microscopic field after the culture period indicated divided by the numbers observed at day 1 in the same field.

cells/ml as described (Tschan et al., 1990). Briefly, cells suspended in 0.5% agarose in DME were seeded into dishes precoated with agarose gels and were allowed to sediment to the interface between the agarose layers. The upper agarose layer containing the cells was then allowed to solidify by brief exposure to 4°C (Benya and Shaffer, 1982). The medium was DME containing 1 mM cysteine. Where applicable, media also contained FBS, recombinant human IGF-I (a kind gift from Ciba Geigy AG, Basel, Switzerland), recombinant human PDGF B/B (Boehringer Mannheim GmbH, Mannheim, FRG), recombinant bovine basic FGF (Progen GmbH, Heidelberg), or thyroxine (Sigma Chemical Co., St. Louis, MO), respectively. All media were replaced after every 2–3 d.

Determination of Cell Proliferation

Cells at the agarose interfaces were monitored by phase-contrast light microscopy. Several representative fields of each culture dish were followed throughout the entire culture period and rates of proliferation of at least 25 cells per field arbitrarily selected at the beginning of the cultures were determined by direct counting on the micrographs.

Synthesis of Collagen and Proteoglycans

Cultures were labeled with 1 μCi/ml of [U-^{14}C]proline (285 mCi/mmol; Amersham International) for 3 d or with 2.5 μCi/ml of ^{35}SO$_4$ (1.209 Ci/mmol; Amersham International) for 24 h. Collagens were extracted from whole cultures after digestion with pepsin (Serva, Heidelberg, FRG) as reported (Benya and Shaffer, 1982). Precipitation of the extracts at neutral pH with (NH$_2$)$_2$SO$_4$ at 45% saturation or with 4 M NaCl yielded radiolabeled peptides derived from collagens, only, as shown by SDS-PAGE (see Fig. 2). Radiolabeled proteoglycans were extracted from whole cultures with 4 M guanidinium hydrochloride, 10 mM EDTA, 50 mM sodium acetate, pH 6.5, and quantified as described (Bruckner et al., 1989).

Table II. Collagen Synthesis as Determined by Incorporation of [^{14}C]proline into Pepsin-resistant Protein

	FBS	cpm/dish ($\times$ 10^{-3}) at day		
		7	14	21
	%			
Experiment 1	0	1.28	1.90	1.03
	0.1	8.42	9.22	12.56
	1	62.64	71.90	66.14
	10	422.62	37.92	57.04
Experiment 2	0	0.76	1.50	1.55
	0.1	8.12	27.48	23.30
	1	304.46	21.08	30.64
	10	626.82	252.64	153.66

Table III. Proteoglycan Synthesis Determined by Incorporation of ^{35}SO$_4$ into Nondialyzable Material

	cpm/dish ($\times$ 10^{-3}) at day			
FBS	4	7	14	24
%				
0	12.920	16.940	17.440	15.960
	18.415	17.550	19.180	14.208
0.1	27.599	77.000	18.720	28.560
	19.306	44.750	21.560	31.640
1	35.893	103.320	120.692	322.128
	33.020	82.460	95.715	322.420
10	99.120	302.330	381.000	158.044
	77.000	422.660	450.808	—

The values indicated were obtained for duplicate dishes of the same cell preparation.

Determination of Alkaline Phosphatase Activity

Whole cultures were extracted for 2 h at 4°C with 25 mM Tris-HCl, 1.25 mM MgCl$_2$, 0.2 mM PMSF, and 0.25% Triton X-100, pH 7.4. Agarose was removed by centrifugation and alkaline phosphatase activity in the supernatants was measured by the method of Bessey et al. (1946). Briefly, the kinetics of hydrolysis of p-nitrophenyl phosphate in 1 M diethanolamine-HCl, pH 9.8, by the extracts were monitored at 405 nm.

For histochemical detection of alkaline phosphatase-activity, 35-mm culture dishes were supplemented with 1 ml of 100 mM Tris-HCl, 100 mM NaCl, 50 mM MgCl$_2$, pH 9.5, containing 10 μl of 30 mg/ml Nitrobluetetrazolium (Sigma Chemical Co.) in 70% dimethylformamide and 10 μl of 15 mg/ml 5-Bromo-4-chloro-3-indolyl-phosphat (X-phosphat; Boehringer Mannheim) in DMF. After 30 min in the dark at 37°C, cultures were subjected to phase-contrast light microscopy.

Results

Proliferation of chick embryo sternal chondrocytes in suspension culture in agarose was determined by counting of individual cells in representative micrographs of the same fields taken after various periods of time. As shown in Fig. 1, this method was easily applicable in early cultures up to two weeks. In older cultures containing FBS, cells formed clusters in which individual cells could not well be discerned.

FBS is not only a powerful mitogen for chick embryo sternal chondrocytes but also induces their maturation. Hypertrophic chondrocytes appear after 7 d in culture with 10% FBS (Bruckner et al., 1989; Tschan et al., 1990). Here, we determined the dose dependence of FBS on the stimulation of cell proliferation and the synthesis of collagens and proteoglycans. In contrast to serum-free conditions, media with only 0.1% of FBS activated proliferation (Table I) and deposition of matrix macromolecules. In such cultures, the quantities of newly synthesized collagens varied from experiment to experiment but were at least five times larger than in serum-free cultures (Table II). Proteoglycan synthesis was less increased (Table III). However, at these low concentrations of FBS cells showed no distinct signs of hypertrophy. They were only slightly enlarged in comparison to their initial size (Fig. 1, A–C) or to control cells (Fig. 1 I) and synthesized neither collagen X (Fig. 2, lanes 1, 4, and 6) nor alkaline phosphatase (Table IV). The cells maintained this state for at least 9 wk (not shown).
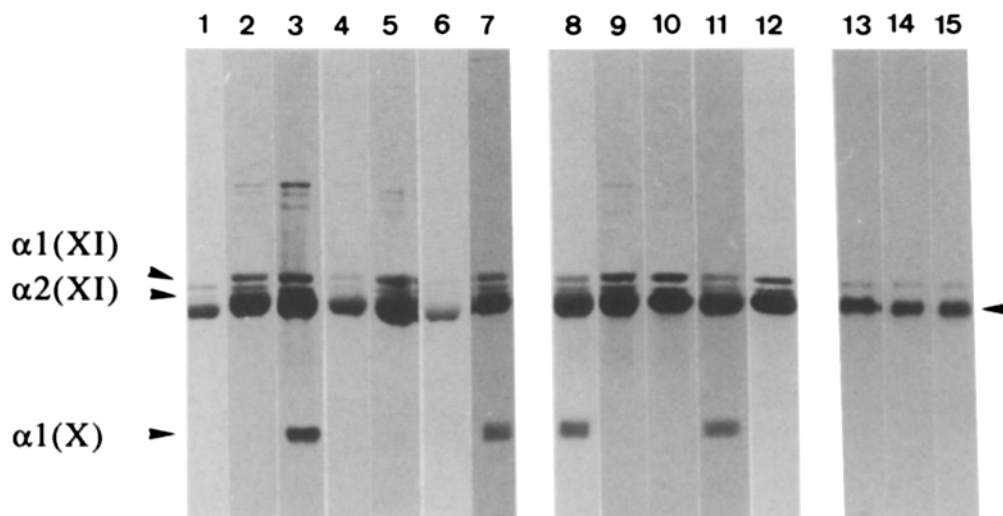
Figure 2. Fluorograph of pepsin-digested collagens metabolically labeled for 3 d with [^{14}C]proline, and subjected to SDS-PAGE (4.5–15% gradient gel). Cells were grown with 0.1% FBS (lanes *1*, *4*, and *6*), 1% FBS (lanes *2*, *5*, and *7*) or 10% FBS (lane *3*) for 7 d (lanes *1–3*), 14 d (lanes *4* and *5*), and 21 d (lanes *6* and *7*). Cells were cultured with 1 nm of thyroxine for 10 d (lanes *8* and *11*), 100 ng/ml of IGF-I for 10 d (lanes *9* and *12*), 100 ng/ml of insulin for 10 d (lane *10*), 1 ng/ml of basic FGF for 8 d (lane *14*), or with 1 ng/ml of PDGF for 8 d (lane *15*). Collagens synthesized by serum-free control cells (lane *13*). Samples in lanes *11* and *12* were reduced with 2% b-mercaptoethanol before electrophoresis.

When the serum content of the media was increased to 1%, cells not only proliferated (Table I), but eventually also became hypertrophic. After 2 wk, they were distinctly larger than control cells (Fig. 1, compare *F* and *I*) and transiently produced larger amounts of matrix molecules. Most importantly, however, the cells produced collagen X increasingly with time in culture (Fig. 2, lanes *2*, *5*, and *7*). As expected, signs of hypertrophy including expression of collagen X appeared later than in cells exposed to 10% FBS (Fig. 2, lane *3*). In response to FBS, cells acquired a somewhat irregular shape, the extent of which depended on length of culture and the amount of FBS (Fig. 1, *C* and *F*).

To identify candidates among serum components responsible for the effects described above, we studied growth factors and hormones in serum-free cultures. As a first example, IGF-I was selected because it is a prominent growth factor of serum (Humbel, 1990), stimulates deposition of extracellular matrix in cartilage explants (Salmon and Daughaday, 1957), has been reported to promote proliferation in chondrocytes in vitro in the presence of FBS (Vetter et al., 1989; Froger-Gaillard et al., 1989; Trippel et al., 1989), and may substitute for growth hormone during development or

growth of hypophysectomized animals (Froesch et al., 1990). IGF-I stimulated proliferation of chondrocytes and increased matrix production, albeit in a manner distinct from that of FBS. Under the direction of 10–100 ng/ml of IGF-I, a range of concentrations expected to exist in media containing 10% FBS (Humbel, 1990), cell numbers increased about threefold after a lag period of 3–10 d, reaching plateau levels around 14–20 d (Figs. 1 *L* and 3). The exact time of onset and of termination of cell proliferation varied between ex-

Table IV. Production of Alkaline Phosphatase in Cultures Treated with Serum, IGF-I, or Thyroxine

	Enzymatic activity at day		
	8	14	21
Serum-free culture	0.010	0.035	0.021
10% FBS	40.000	62.000	75.000
0.1% FBS	0.045	0.035	0.021
100 ng/ml IGF-I	0.011	0.070	0.021
100 nM Thyroxine	ND	127.000	41.000

Enzymatic activity was measured as micromoles *p*-nitrophenol per minute per dish liberated from *p*-nitrophenyl phosphate.
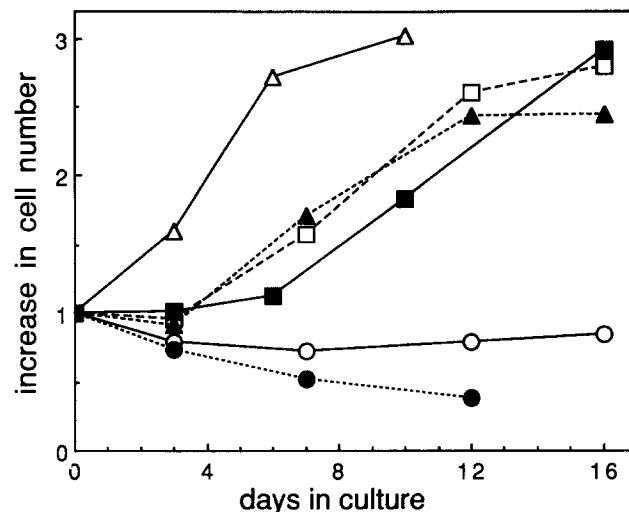

Figure 3. Cell proliferation of chondrocytes under the direction of 10% FBS (△), 10 ng/ml (□) or 100 ng/ml of IGF-I (▲), 100 ng/ml of insulin (■), or 1 ng/ml of thyroxine (●). Control cells (○). Light micrographs were repetitively taken from the same field during the experiments. The increase of cell numbers corresponds to the number of cells counted at the day indicated divided by the numbers observed in the same field immediately after inocculation. Results of a typical experiment are shown.
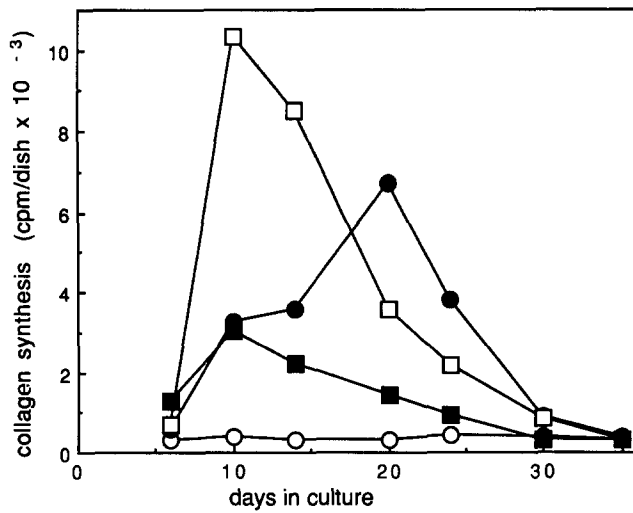
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Figure 4. Amount of collagens newly synthesized by cells under the influence of IGF-I. 10 ng/ml (●), 100 ng/ml (□), or 1 μg/ml (■). Control (○). Collagen production was determined by incorporation of radioactively labeled proline into pepsin resistant macromolecules (for details, see text). Results of a typical experiment are shown.

Figure 6. Level of proteoglycan synthesis in IGF-I treated cells. The amount of newly synthesized proteoglycans was determined as $^{35}SO_4$ incorporated into nondialyzable material extracted from cultures with 4 M guanidinium chloride. (*Shaded columns*) 10 ng/ml; (*hatched columns*) 100 ng/ml; (*filled columns*) 1 μg/ml; (*open columns*) control cells.

periments and closely correlated with the long-term viability of control cells cultured without growth factors. Importantly, however, the lag period was not observed in cells treated with 10% FBS (Fig. 3), demonstrating the capability of the cells to proliferate immediately as a result of an appropriate stimulus. IGF-I treatment led to a strong transient increase in the level of collagen production (Fig. 4). Interestingly, collagen synthesis increased and subsided simultaneously with cell proliferation (Fig. 5). Cells exposed to IGF-I synthesized collagens II, IX, and XI, but neither collagen X (Fig. 2) nor alkaline phosphatase (Table IV), even after long culture periods. In agreement with its early designation as sulfation factor, IGF-I augmented secretion of proteoglycans (Fig. 6) in parallel with collagen production. Therefore, IGF-I did not change the chondrocytic phenotype and, in contrast to FBS, did not promote hypertrophy of chondrocytes derived from whole 17-d chick embryo sterna.
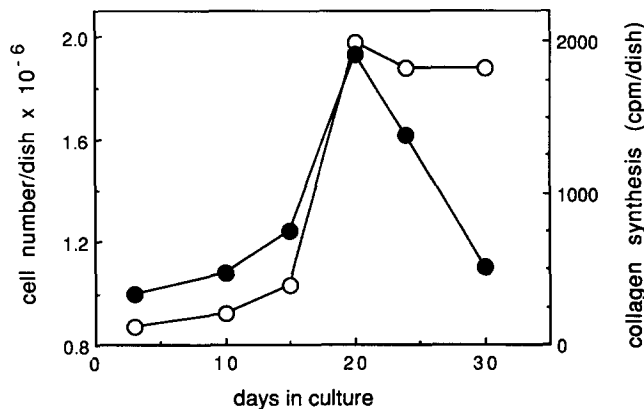

Figure 5. Synchronized proliferation and collagen production of cells under the influence of 10 ng/ml IGF-I. Production of pepsin-resistant protein (●); cell proliferation (○). Conditions were as described for Figs. 3 and 4.
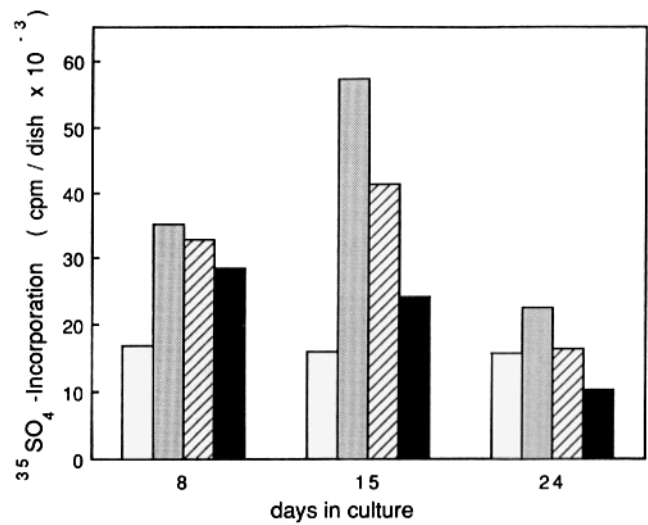
Because of the partial cross-reactivity of insulin with the receptor of IGF-I, we predicted that it could mimic IGF-I-activity at 10–100 times larger concentrations. As shown in Fig. 3, however, insulin and IGF-1 induced proliferation at similar concentrations. Collagen synthesis was stimulated by 100 ng/ml of insulin and occurred at elevated levels even after cell proliferation ceased (Fig. 7). We concluded that insulin acted primarily via its own receptor rather than that of IGF-I. Collagens II, IX, and XI, but not collagen X, were secreted in response to 100 ng/ml of insulin (Fig. 2, lane *10*).

Basic FGF has been designated as the most potent chondrocyte mitogen in vitro (Kato et al., 1987). In addition, Iwamoto et al. (1991) recently reported that basic FGF inhibited chondrocyte hypertrophy in culture. In the present investigation, basic FGF without serum was a very poor mitogen. Instead, the cells only slightly changed their shape after 2 wk in the presence of 0.1–10 ng/ml of this growth factor (Fig. 1 *J*), but neither proliferated (Fig. 1, compare *I* and *J*) nor generated more collagen than control cells (Fig. 2, compare lanes *13* and *14*). The pattern of collagen expression remained unchanged (Fig. 2, lane *14*). In conjunction with 10% FBS, basic FGF stimulated colony formation of cells at an initial density of 2 × 10⁴ cells/ml (not shown) as reported (Kato et al., 1987).

PDGF accounts for a large fraction of the mitogenic activity of serum (Ross et al., 1986) and chondrocytes express receptors for PDGF (Bowen-Pope et al., 1985). Under the present culture conditions, this factor did not promote cell division, matrix synthesis, or chondrocyte hypertrophy. Results closely comparable to those with basic FGF were obtained with 1–100 ng/ml of PDGF (Fig. 1 *K* and Fig. 2, lane *15*).

Thyroxine has well known effects on skeletal development. In man, chronically low levels of thyroxine during fetal development and in infancy are associated with growth retardation (skeletal cretinism) and elevated levels with accelerated skeletal development (Schlesinger and Fisher, 1951). Fur-
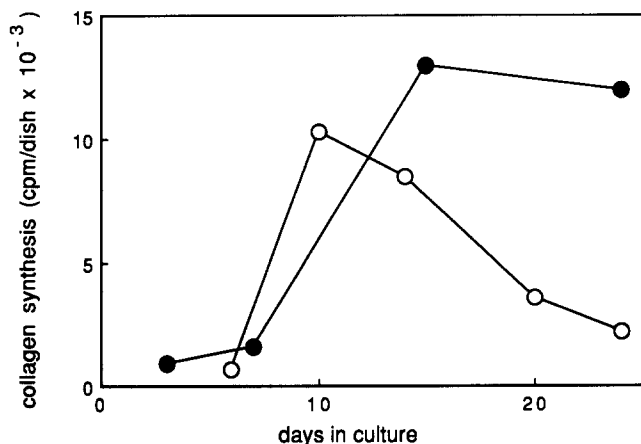
Figure 7. Collagen production under the control of 100 ng/ml of insulin (●) or 100 ng/ml of IGF-I (○). Experimental conditions were as described in Fig. 4. Note: Collagen synthesis continues at elevated level in cells treated with insulin but not IGF-I after cessation of cell division.

thermore, the hormone has been reported to support chondrogenic expression in monolayer cultures (Pawelek, 1969). Under our conditions, thyroxine affected remarkably the phenotype of chondrocytes in vitro. When the media contained 1–100 nM of the hormone, cells decreased in numbers (Fig. 3), but drastically increased their size between day 7 and 14 in culture (Fig. 8 A). Strikingly, the pattern of newly synthesized collagens also included collagen X (Fig. 2, lanes 8 and 11). Consistent with the behavior of hypertrophic chondrocytes, some of the cells secreted large amounts of alkaline phosphatase (Table IV) conspicuously associated with matrix vesicles (Fig. 8 A).

Discussion

The cells used in this study were derived from entire 17-d chick embryo sterna and, therefore, represented a heterogeneous population of resting, proliferating, and, possibly, early hypertrophic chondrocytes. However, fully mature chondrocytes synthesizing collagen X and alkaline phosphatase were absent since they appear in the organ only at day 18 (LuValle et al., 1989). Because the tissue surrounding the sterna can easily be removed, cells other than chondrocytes were also not present. Our isolation and culture protocols (Tschan et al., 1990) yielded at least 85% viable cells with properties similar to those in situ at the beginning of the culture experiments. We have employed this cell population as a tool to identify agents involved in the induction of chondrocyte hypertrophy. Because our cultures contained cells at different stages preceding hypertrophy we anticipated that, at least in a part of the cells, extracellular signals would have elicited responses if they played a role in terminal chondrocyte differentiation in situ.

The mitogenic activity of FBS remained unmatched by all agents tested. Very small amounts of serum triggered cell division, underscoring again the necessity of serum-free culture conditions in studies on regulatory effects of extracellular signals on chondrocytes. Hypertrophy, however, is not induced at very low levels of FBS even after long culture periods. A striking variability of the proliferative and meta-
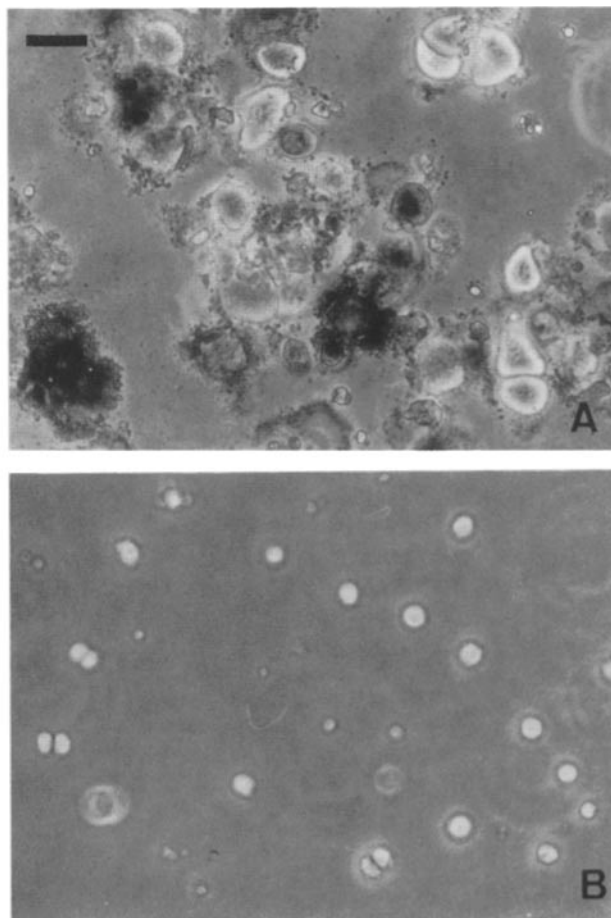

Figure 8. Phase-contrast micrograph of cultures stained for alkaline phosphatase-activity. (A) Cells treated with 1 nM thyroxine for 12 d. Note: Matrix vesicles are heavily stained. (B) Control cells. Bar, 25 μm.

bolic responses of the cells to FBS was observed between experiments. The reason for this is unknown, but may well result from the complexity of cellular responses to the diverse mixtures of signaling molecules in FBS, further emphasizing the need for strictly controlled culture conditions in studies on environmental regulation of cell functions. In our earlier studies, we have described culture conditions for resting or hypertrophic chondrocytes (Bruckner et al., 1989; Tschan et al., 1990). Here, we have established conditions for the maintenance of cells resembling those of the proliferative zone of developing long bones or the sternum of chick embryos.

IGF-I and insulin were mitogenic to chondrocytes independently from each other. In both cases, no additional factors were required. In response to IGF-I at physiological or lower concentrations, cell numbers reached the same plateau level as in the presence of 10% FBS. This is consistent with the notion that IGF-I was a major serum-mitogen for chondrocytes. However, IGF-I and 10% FBS differed from each other in that proliferation was induced by IGF-I only after a lag period. We concluded that proliferation stimulated by FBS does not result from the exclusive action of IGF-I. In addition, chondrocytes probably became sensitive to IGF-I only through synthesis of additional components.

Likely candidates include synergistic growth factors, IGF-binding proteins (Sara and Hall, 1990), or matrix components. It is also possible that IGF-I-receptors enzymatically removed from cell surfaces during preparation were reconstituted during the lag period.

In contrast to IGF-I or insulin, basic FGF and PDGF neither promoted cell division nor matrix production nor hypertrophy in serum-free cultures of chondrocytes. However, basic FGF bolstered the mitogenic activity of FBS in cultures with low cell densities, indicating that the cells became conditioned by FBS so that they could respond to basic FGF. It is probable, however, that chick embryo sternal chondrocytes in situ are not sensitive towards basic FGF. Indeed, basic FGF acted only on injured cartilage in vivo where it could elicit repair but was inactive towards intact tissue (Cuevas et al., 1988).

Thyroxine at or below concentrations found in normal chicken plasma (Scanes et al., 1989) proved to be a strong initiator of chondrocyte hypertrophy. Preliminary results suggested that triiodothyronine had a similar activity. Again, no synergism with other components was needed. These observations were predicted by the study of Burch and Lebovitz (1982a,b) reporting that triodothyronine and thyroxine stimulated growth and maturation of embryonic chick cartilage in serum-free organ culture. The presence of collagen X produced by our cells treated with thyroxine definitively corroborates this notion. However, our conclusions differ from those of Kavumpurath and Hall (1990) who did not find chondrocyte hypertrophy in embryonic chick Meckel's cartilage in response to thyroxine. Because hypertrophic chondrocytes in mammalian growth plates always originate from cells that have previously proliferated in columnar patterns, it has been assumed that proliferation was a necessary step preceding terminal differentiation. Support for this concept has also come from in vitro studies on chondrocyte maturation (Castagnola et al., 1988; Quarto et al., 1990). However, in secondary centers of ossification, chondrocytes normally proceed towards hypertrophy without extensive proliferation. Depending on the culture conditions, our in vitro system is strikingly capable to duplicate both pathways of endochondral ossification in vivo. In response to thyroxine, cell proliferation was not required prior to terminal differentiation.

Hypertrophy of chondrocytes is a normal process in growth plates where mature cells prepare the tissue for ossification. By contrast, terminal differentiation of chondrocytes resulting in the production of collagen X occurs at low level and only in deep zones of normal articular cartilage where the tissue is permanent (Gannon et al., 1991). However, in degenerative joint disease, brood capsules of proliferating cells are found near fissures at delaminating articular surfaces (Sokoloff, 1979). Interestingly, collagen X is also present at highly elevated level in such tissue (von der Mark and Glückert, 1990) indicating that pathologically altered articular cartilage has acquired the ability to respond to stimuli initiating terminal chondrocyte differentiation. It is tempting to speculate that some of the signaling molecules studied here may play an important role not only in endochondral bone formation but also in degenerative joint diseases.

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