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Direct demonstration of production of transforming growth factor activity by embryonic chick tissue

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Summary. The presence of transforming growth factor activity in early chick embryos was directly demonstrated by the ability of limb and tail buds to induce anchorage independent division in NRK 49 f cells. Colony number increased with limb bud number and developmental stage. Medium conditioned by tail buds contained some stimulating effect, and strongly promoted the action of other transforming growth factors.
Key words. Transforming growth factor; chick embryo; limb bud.

Although it is possible to isolate transforming growth factors [1] from embryos [2-6] it is not yet clear what function they normally fulfil. Two problems arise with the standard procedure. One is that to obtain enough starting material, embryos are usually homogenised, losing information about spatial distribution of the factors. A second is that only known transforming growth factors can be readily identified when present in small amounts, so unknown transforming growth factors may not be detected. An alternative method which avoids both these problems is to test the ability of pieces of embryonic tissue directly for their ability to induce colony formation by fibroblasts grown in soft agar. We selected chick embryo limb buds as the test tissue, since chick embryos are easy to obtain at predetermined stages, and the limb buds can be removed in a consistent manner from embryo to embryo. There is already evidence that limb buds produce mitogenic growth factors [7, 8].

Fertile cross-bred hens eggs (Muirfield Hatcheries, Kinross) were incubated for periods of 4–5 days at 39 °C, then opened and staged by the criteria of Hamilton and Hamburger [9]. Embryos were removed into 30-mm dishes of Hams F12 medium containing 5% foetal calf serum (Gibco). Limb buds were removed by a cut across the proximal margin at the body wall, using electrolytically sharpened tungsten needles. Tail buds were removed by cuts transverse to the neural tube, at a distance equivalent to the width of the tail. Buds were washed once by transferal to fresh medium. Conditioned medium was prepared by culturing groups of 3 tail buds in 0.3 ml serum-free medium for 24 h, after which the medium was removed and made up to 1 ml with serum-free medium.

NRK clone 49f cells (a gift from Dr Austin Smith, Department of Zoology, Oxford) were cultured in Hams F12 containing 5% foetal calf serum (FCS) and passaged at 3–4-day intervals. Equal volumes of 0.6% Bacto-agar (Difco) and 2× Dulbecco's MEM (Flow Laboratories) with 22.4% FCS containing 4×10^3 NRK 49f cells per ml were mixed together at 40 °C. At final concentration the medium also contained 50 IU/ml penicillin and 50 µg/ml streptomycin (both Flow Laboratories). 1 ml of this mixture was added to a 30-mm bacteriological grade plastic petri dish (Sterilin). Limb and tail buds were placed in petri dishes prior to addition of the cells. Buds were transferred using handmade tantalum foil trays. EGF (Sigma), TGF-β (Peninsula Laboratories), conditioned media and control media were added to the dishes in 0.24-ml volumes.

Colony number was assessed 9 days after the start of each experiment. The scoring criterion was that a colony must contain four or more cells, as observed using an inverted microscope. No colonies were observed in controls lacking limb buds or additional growth factors, in controls in which the soft agar had been stirred with forceps previously used to handle embryos, or in controls where yolk particles were added to the dish. However, colonies were observed in the presence of limb buds, and the number of colonies increased with the number of limb buds (fig. 1). 3 tail buds induced colonies at a similar frequency to one limb bud. The effect of developmental stage was investigated by assaying the number of colonies induced by the presence of 2 wing buds per dish, at stages 18–26, and it was found that the number of colonies tended to increase with developmental stage (fig. 2),

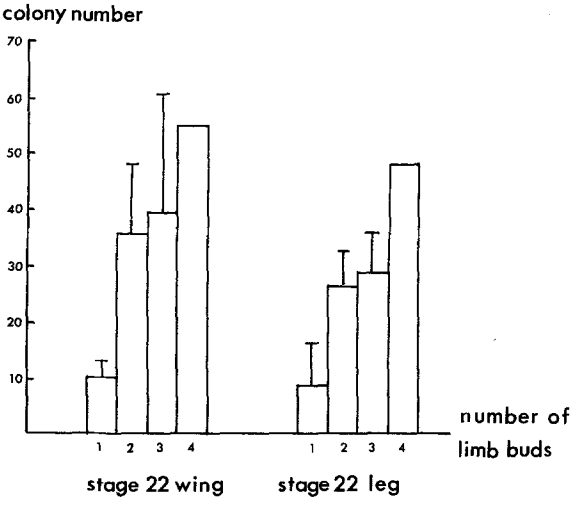

Figure 1. Effect on colony number of increasing number of wing and leg buds. Each value is the mean of between 6 and 8 determinations, except those involving 4 limbs, which were determined once only. Error bars represent standard deviations.

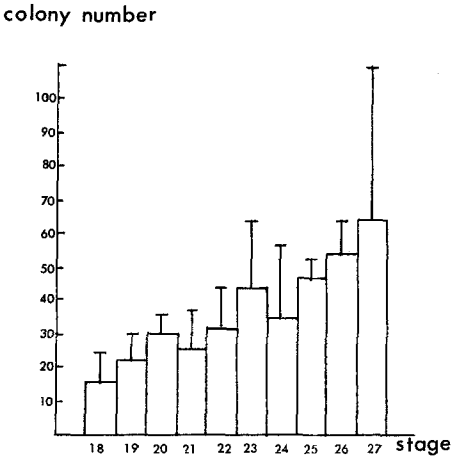

Figure 2. Effect on colony number of increasing developmental stage. 2 wing buds were cultured in each dish. Each value is the mean of between 3 and 10 determinations. Error bars represent standard deviations.

Effect of additional growth factors on stage 19 tail bud conditioned medium. Values given are the mean number of colonies formed over five determinations, followed by the standard deviation in brackets. Two similar experiments gave the same pattern of outcome.

	No additives	4 ng/ml EGF	2 ng/ml TGF-β	4 ng/ml TGF + 2 ng/ml TGF-β
Control	0 (0)	322 (26)	2 (1)	467 (51)
Conditioned	7 (3)	356 (25)	5 (2)	749 (35)

though there was some variability. During this time, however, the size of the limb bud is also increasing markedly [10], so relative transforming ability per µg protein is either remaining constant or falling. Tail bud conditioned medium induced formation of colonies (table). Addition of TGF-β (final volume 2 ng/ml) to conditioned medium had little effect. Addition of EGF (final volume 4 ng/ml) to conditioned medium led to a non-significant increase over controls with the same EGF level. Addition of both EGF and TGF-β to conditioned medium leads to a significant increase in colony number over the equivalent controls ($p < 0.001$ by t-test analysis).

The responsiveness of the assay system to fixed doses of EGF varies from day to day and it is therefore difficult to establish standard dose response curves which give exact results. However, 2 limb buds have an equivalent effect to the presence of EGF in the range 0.01–0.05 ng/ml. If colony stimulation by conditioned medium was entirely due to an EGF-like molecule at these doses, addition of TGF-β should enhance it [11], but this was not the case (table). From these results, the conditioned medium could contain small amounts of both EGF-like activity and TGF-β like activity, or molecules with so far unidentified interactive characteristics.

The technique described here opens up the possibility of mapping a single embryo for its distribution of transforming growth factors, using only the simplest equipment. It is hoped that this procedure will reveal local sites of production, and hence suggest roles for these molecules in development.

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Variability of β-amylase isoenzymes within a collection of inbred lines of rye (*Secale cereale* L.)

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Summary. Within one collection of 18 inbred rye lines, seven distinct isoenzymatic patterns of β-amylase were found, and five of them formed a group of similar patterns shifting to distinct positions in the gel. It was proved that the shift of the pattern was due to genetic factors. In crossed immunoelectrophoresis, drastic differences were shown in the quantity of the different β-amylase constituents. No antigenic differences could be demonstrated between the analyzed isoenzymes.
Key words. Secale cereale; β-amylase; variability; immunochemical characteristics.

β-Amylase, isolated from mature kernels of cereals such as hexaploid wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) exhibits a significant polymorphism. Foundations of the latter have not been clarified completely; as suggested by available literature data, they may involve numerous structural genes [1–3] and also post-translational modifications [4–6].

In this report we describe the β-amylase isoenzymatic variability within a collection of inbred lines by means of isoelectric focusing and crossed immunoelectrophoresis. The report