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Control of translation of globin mRNA in embryonic cells

WHEN mouse or rabbit globin mRNA is injected into oocytes of *Xenopus laevis* both α and β mRNAs are translated but only about one fifth as much α globin is synthesised as β globin[1–3]. On the other hand, the same message sample results in about equal amounts of α and β globin synthesis in a reticulocyte cell-free system and is believed to contain roughly equal amounts of α and β globin mRNA. Giglioni *et al.*[3] have shown that if haemin is injected into oocytes at the same time as globin mRNA, there is an equal ratio of $\alpha:\beta$ globin synthesis, and their experiments strongly suggest that this is achieved by increasing the efficiency of translation of α globin mRNA. These experiments thus identified a factor which has a different effect on two kinds of mRNA, and further demonstrated that the microinjection of living cells can reveal regulatory effects not readily seen in the cell-free systems used so far. The experiments reported here were also carried out by injecting mRNA into frog cells. They show that during development a component is formed that alters the ratio of $\alpha:\beta$ rabbit globin synthesis, a change not seen when mouse globin mRNA is injected. This is therefore a second example of a translational factor revealed by a living cell assay system. This component shows an even higher degree of selectivity in its action than haemin.

Rabbit or mouse globin mRNA is translated into globin when injected into fertilised frog eggs[4–6], which differ in several respects from oocytes. Mouse mRNA yields a low, although somewhat variable, ratio of α to β globin synthesis in eggs and embryos of all stages (Table 1), as it does in oocytes (Fig. 1). There is no proof that the extremely low mouse $\alpha:\beta$ ratios seen in eggs and morulae are significantly different from the low ratios seen in other stages. Rabbit globin mRNA also yields a low ratio of $\alpha:\beta$ globin when translated in unfertilised or newly fertilised eggs; on the other hand, this ratio changes progressively, in the absence of injected haemin, to an almost equal synthesis of α and β globin as the embryos develop (Table 1). The ratio of $\alpha:\beta$ globin synthesis approaches one, when tested in embryos at and beyond the neurula stage (20 h after fertilisation, Table 1).

Haemin can restore the efficient translation of rabbit and mouse α-globin mRNA in oocytes; but unlike the *Xenopus* embryo factor, it is equally effective with mouse and rabbit globin mRNA (Fig. 1). For this reason the *Xenopus* factor is probably not itself haemin, though it may operate by part of the same mechanism since haemin and the embryo factor seem not to have an additive effect on rabbit globin synthesis in neurulae.

Some information is available as to why less α globin is made than β globin. For example this effect does not result from differential breakdown of completed α chains because α and β peptides are present in the usual 1:5 ratio in the polysomes of mRNA-injected oocytes[7]. It also cannot be explained in a simple way by destruction of α rather than β mRNA because haemin increases the level of α-globin synthesis even when injected as long as 48 h after the mRNA (Table 2). It seems likely therefore that the effect is a result of a true translation control phenomenon.

Two explanations of the haemin effect seem plausible. One is that haemin raises the efficiency of initiation of α but not β-globin mRNA. Alternatively haemin might re-

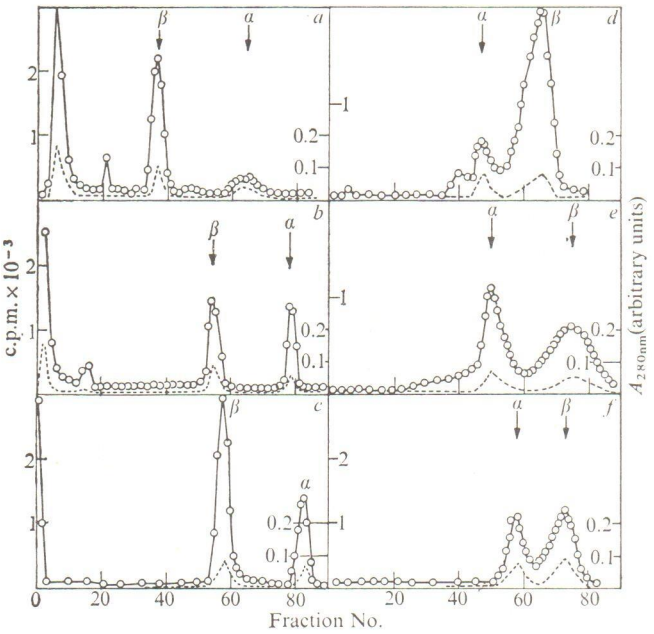

Fig. 1 Effect of haemin on globin synthesis in oocytes injected with saturating and subsaturating amounts of rabbit and mouse globin messengers. A stock of 16 mM haemin was prepared by the method of Zucker and Schulman[10] except that the final solution contained 90% (v/v) ethylene glycol. Oocytes were injected with mRNA dissolved in saline[1], the latter containing haemin (1.6 mM) where indicated: the intracellular concentration of haemin would have been about 80 μM. Globin was prepared from whole oocytes and was fractionated by carboxymethyl cellulose chromatography[2]. O——O, c.p.m. per fraction; – – –, absorbance at 280 nm of added marker α and β globin chains. *a*, globin synthesis ($\alpha:\beta=17\%$) in oocytes injected with mouse mRNA (12 ng per cell); *b*, the effect of haemin ($\alpha:\beta=84\%$) on synthesis directed by such subsaturating amounts of mouse mRNA; *c*, the effect ($\alpha:\beta=46\%$) of haemin on saturating amounts (95 ng per cell) of mouse mRNA; *d* ($\alpha:\beta=12\%$) and *e* ($\alpha:\beta=84\%$) (+haemin) show analogous experiments with subsaturating rabbit mRNA (24 ng per cell), while *f* shows globin synthesis ($\alpha:\beta=55\%$) with saturating rabbit mRNA (48 ng per cell) plus haemin. The $\alpha:\beta$ globin synthesis ratio ($\alpha:\beta=20\%$) in oocytes injected with saturating messenger and no haemin is similar to that seen in oocytes injected with subsaturating mRNA. Oocytes injected with buffer containing no RNA synthesise, no globin like species.

Table 1 Ratio of α:β-globin synthesis in mRNA-injected oocytes and eggs

Hours after fertilisation	Oocyte (ovarian)	Oocyte (matured)	Unfertilised egg 0	Morula 4	Blastula 8	Gastula 14	Neurula 20	Post-neurula 26	Early tail bud 44	Tail bud 50	Heart beat 54	Swimming tadpole 72	Stage 45 180
Mouse globin mRNA	20	—	3	5	24	10	10	20	14	15	11	11	—
Rabbit globin mRNA	17	20	19	22	67	59	60	68	66	—	—	54	54

Figures show ^{3}H-α-globin radioactivity as a percentage of ^{3}H-β-globin synthesis. Oocytes and eggs were injected with mouse (12 ng per cell) or rabbit (7–20 ng per cell) globin mRNA[1,5]. After the appropriate time, cells were labelled with ^{3}H-histidine by incubation (oocytes) or injection (embryos). Embryo homogenates were treated with acid acetone, and the resulting globin was chromatographed on a carboxymethylcellulose column[2]. Matured oocytes were obtained by progesterone treatment (10 μg ml^{-1}) of immature oocytes.

move some restriction to ribosome transit[7] located about half way along the α-globin mRNP particle. Neither model necessitates a requirement for specific factors in the translation of specific messages and both models can be used to describe the agent enhancing rabbit α-globin synthesis during development. Since haemin and the developmental factor differ in their effects on mouse globin synthesis, however, their mechanism of action cannot be identical. The significance of these two regulatory phenomena is not clear. In man, genetic deficiencies in haem synthesis are commonly associated with low globin synthesis and a low (0.71) ratio of α: β-globin synthesis[8,9]. Haem may therefore be involved in α-globin mRNA translation in normal reticulocytes. The principal importance of the embryo factor, whose developmental significance is unknown, is that it indicates the possible existence in cells of a wide range of natural substances which may have very selective effects on message translation; the purification of those may help to reveal the kinds of mechanism by which translation can be controlled.

Table 2 Effect of haemin when supplied after the initial injection of globin mRNA

Haemin injection (h after message)	Period of labelling (h after message)	α:β (as a %)
No haemin	1–16	17
0.15	1–16	93
Simultaneous	1–16	84
No haemin	48–64	25
Simultaneous	48–64	83
48	48–64	57

Oocytes were injected with mouse globin mRNA (12 ng per cell) and were then injected with saline[1] or haemin. The cells were labelled with ^{3}H-histidine. Globin was prepared and fractionated[2]. A further experiment was carried out by labelling oocytes for 5–18 h, with similar results to the 48–64 h experiments shown, except in the case of 'no haemin' samples. About 30 ml of a 1.6 mM solution of haemin was injected per oocyte so as to give an intracellular concentration of about 80 mM.

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