Review Letter

HISTONE SYNTHESIS DURING THE DEVELOPMENT OF *XENOPUS*

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1. Introduction

The chromatin of animal cells contains approximately equal masses of DNA and histone. In the division cycle of a typical eukaryotic somatic cell this equivalence is achieved by close coupling of histone and DNA synthesis. The first aspect of the coupling is temporal: almost all histone is made during the S-phase. Accordingly, histone mRNA is present during the S-phase and most appears to be degraded at its end [1–5]. However, small amounts of histone, particularly H1, may be made at other stages, and the amount may be significant in non-dividing cells (e.g. [6–8]) and especially in late erythropoiesis [9]. Since chromatin contains equal masses of DNA and histones this represents turnover, particularly of the H1 fraction. The second aspect of the coupling is quantitative; because very little free histone can be detected in S-phase cells, the S-phase cell must make the same mass of DNA and histone [10,11].

This tight coupling does not apply to the embryos of the anuran amphibian *Xenopus laevis*. At 23°C its zygote divides to form 30 000 cells in the 9 h period between fertilization and the gastrula stage. After the first cell cycle of 1.5 h, in which the S-phase lasts 20 min and there are recognisable G1 and G2 periods, the cell cycle may be as short as 12 min, with an S-phase of as little as 10 min and no recognisable G1 or G2 [12,13]. Tight regulation of histone synthesis by transcriptional control is not possible over such a short time scale without enormous reiteration of the histone genes. The embryo solves this problem in a different way.

2. Rates of histone synthesis through early development

Rapid histone synthesis during the cleavage of *Xenopus* embryos has been reported by a number of authors [14–17]. The first quantitative study was made by Adamson and Woodland [18]. When absolute rates of histone synthesis were computed, they showed that at early stages the rate of histone synthesis is far in excess of that of DNA (fig.1A). In the fertilized egg histones are made at ~2500 pg/h, whereas the cell replicates only 6 pg of nuclear DNA during the first 1.5 h cell cycle. There is also temporal uncoupling of DNA and histone synthesis, since histones are made at an approximately constant rate throughout this cell cycle, even during mitosis [19]. By the late blastula stage the rate of DNA synthesized per embryo is ~10 000-fold greater than at the single cell stage, but the rate of histone synthesis has only increased 2–3-fold (fig.1). The pattern described above applies to the nucleosomal histones. In contrast the H1 group of histones are made at a low rate until the early blastula stage (500 cells), but then increase to normal levels by the gastrula stage (30 000 cells) [18–20].

Although the amount of histone synthesized after fertilization exceeds that of DNA before the 1000 cell stage, subsequently there is a deficit (fig.1B). To make good this deficiency the egg should contain a pool of at least 140 ng of histone, made previously during oogenesis. Direct measurements revealed a pool of ~135 ng [21], accumulated by synthesis of 50 pg/h in the oocyte [18]. This is sufficient to assemble over 20 000 nuclei, exactly the amount necessary to make good the calculated histone deficit at the beginning of gastrulation. Thus it would seem that, within the limitations of the measurements, the oogenetic pool of histones should be exhausted by the gastrula stage.

The existence of stored histones has been confirmed by an independent method. Laskey et al. [22] showed that egg extracts could assemble ~80 000 pg

of DNA into nucleosomes using endogenous histones. Although they found a lower capacity of the egg to assemble chromatin than was suggested by direct measurement of the histone pool [21] their data was not corrected for recovery.

There would seem to be problems to synthesise DNA in the presence of this vast amount of histones; in vitro excess histone inhibits nucleosome assembly [24–26]. According to Laskey et al. [23] the embryo solves the problem through accumulating a large, negatively charged, heat stable protein in 2-fold mass excess over histones [24]. This protein binds histone and acts catalytically in assembling nucleosomes in vitro. Presumably this is at least one of its functions in vivo.

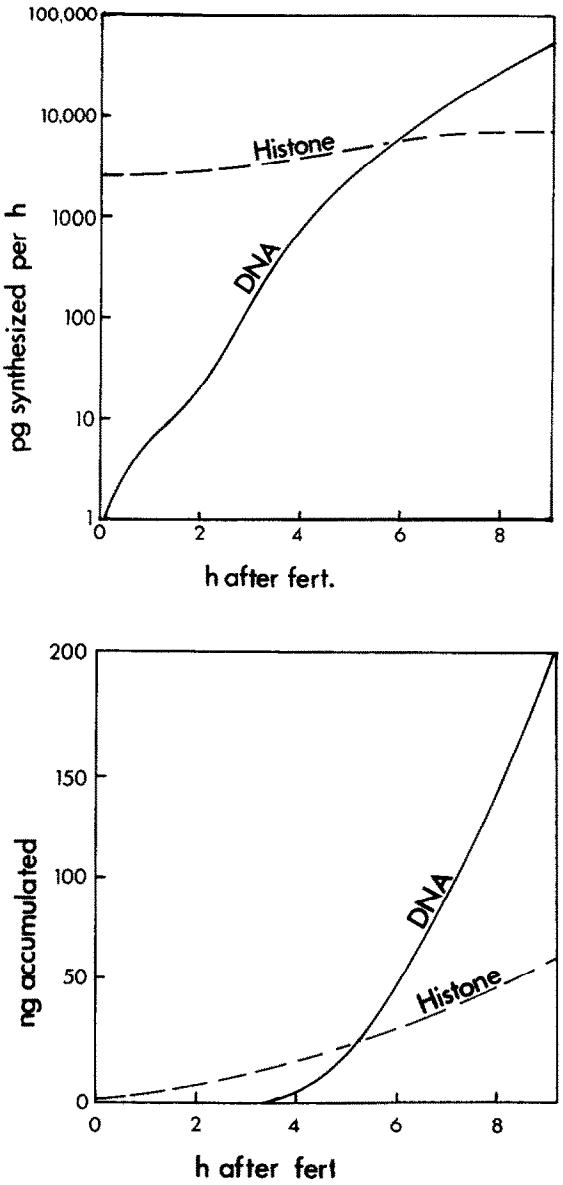

Fig.1. (A) Rates of DNA and histone synthesis from fertilization to the 30 000 cell stage. The rates are on a per embryo basis and are calculated from the data in [18]. (B) Accumulation of newly-synthesized histone over the same period as in (A) (from the data in [18]).

In the oocyte histones are found in the cytoplasm, but they are more highly concentrated in the nucleus [18,21], as predicted from ^{125}I-labelled histone injection experiments [27,28]. This is also true of the heat stable protein [24,29]. The vast nucleus of the oocyte (200–300 μm in diameter) may be a general store for nuclear proteins, since it also contains a stock-pile of RNA polymerases sufficient to support development to the 100 000 cell, tail-bud tadpole stage [30,31]. In terms of the general mechanism of cell determination it would be most interesting to know if the oocyte nucleus contains other stored nuclear proteins, and if any are unequally distributed among cells during cleavage of the embryo.

Some of the histones are 'frozen' in a modification state [32] normally characteristic of newly-synthesized histone before incorporation into chromatin (e.g. [33]). In particular the H4 stored in the nucleus is diacetylated and that in the cytoplasm is probably monoacetylated and monophosphorylated. The extra acetyl groups of the diacetyl nuclear species are removed when the histone is incorporated into chromatin during cleavage. This may be months or years after synthesis, although the acetyl groups also turn over during storage [32].

Oocytes synthesise H1 roughly in proportion to the other histones [20] and presumably the egg therefore contains a pool of H1. This is consistent with the even spacing of nucleosomes seen in chromatin reconstruction experiments [22]. However, H1 synthesis does not accelerate until the blastula stage, although in the gastrula the proportion synthesised has reached the normal level [15,17,18]. It is not clear why H1 and the other histones behave differently. A literal interpretation of the data indicates that late blastula chromatin should be ~30% deficient in H1 histone. Although this conclusion may not be justified, cleavage nuclei are indeed large structures with a diffuse appearance [34]. This is what is expected of H1-deficient nuclei, since removal of H1 is known to decondense chromatin [35]. Against this interpreta-

tion is the fact that embryonic nuclei are largest at the very earliest stages, and become smaller as the stored H1 runs out. H1 deficiency may not be the cause of the unusual nuclear morphology. The embryo contains large stores of nuclear proteins (enough to fill the 300 μm oocyte nucleus), and these may enter the embryonic nuclei, causing them to be swollen until the store is partitioned into many nuclei.

Though the early embryonic nuclei are large, it may easily be calculated that in eggs and cleaving embryos most of the histone and RNA polymerase must be present in the cytoplasm. This means that, as regards protein composition, the entire egg is like a large nucleus. This may account for unusual features of embryonic metabolism, such as RNA polyadenylation in sea urchin eggs (see [36]).

3. Translational control of histone synthesis

The overall pattern of histone synthesis from late oogenesis to the gastrula stage of development is shown in fig.2. The most abrupt change in histone synthetic rate occurs during hormone(probably progesterone)-induced breakdown of the oocyte nuclear membrane, i.e., at the time when the protracted first meiotic prophase ends, converting the oocyte to an egg. The change in overall polysome content is small at this stage (fig.2 and [37]), indicating that the overall rate of protein synthesis rises only slightly. This conclusion is supported by incorporation studies [38] (also calculatable from data in [18]).

Actinomycin D and enucleation experiments have shown that the increase in histone synthesis at matu-

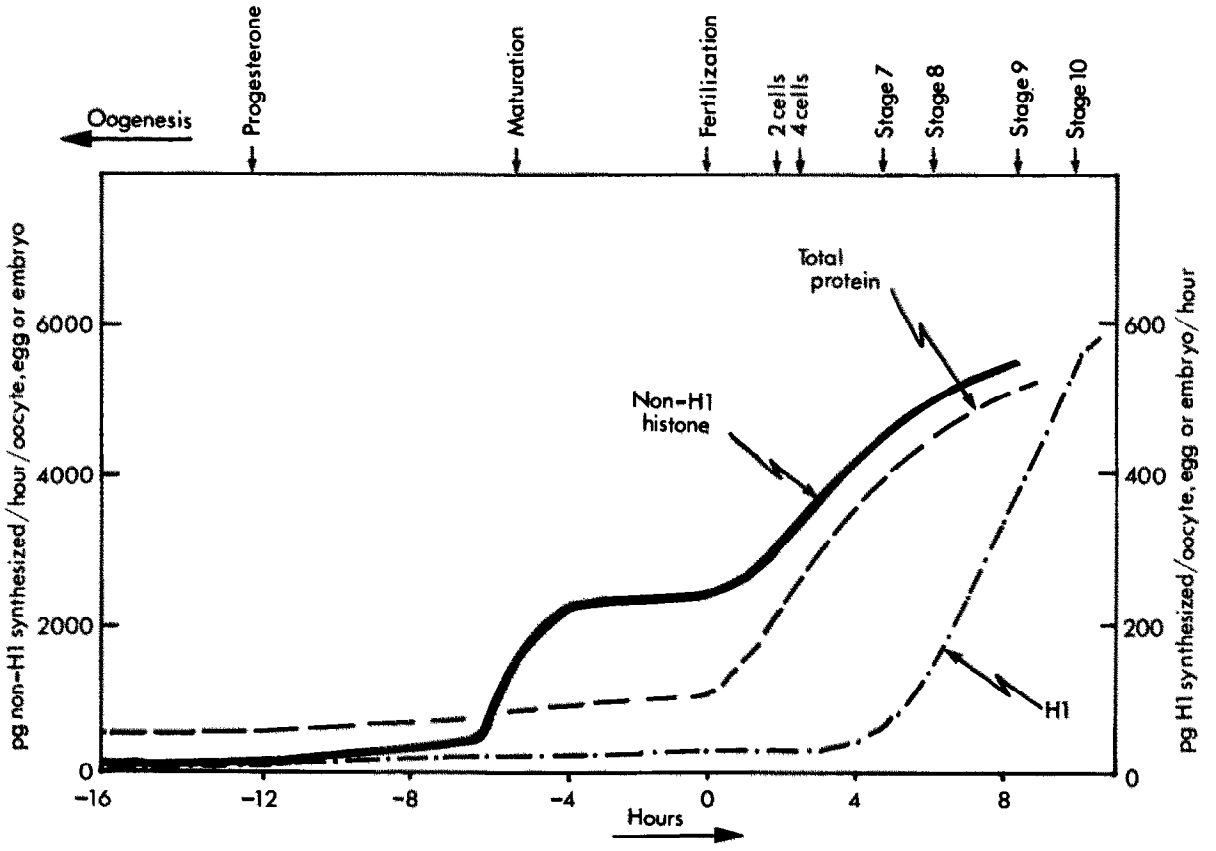

Fig.2. The relationship of histone synthetic rates and total protein synthesis during late oogenesis (a period of several months) and early development up to the 30 000 cell stage. The total protein synthesis rate is presented on an arbitrary scale and represents the polysome content ([37] see also [19]). The rates of H1 synthesis are taken from [20], and those of the non-H1 histones from [18] and [19], although the stage 9 rate is revised downwards in accordance with the data in [20].

ration is brought about by post-translational control [19], and as expected the histone mRNA content, as judged by template activity in vitro, changes little between the oocyte and late blastula stages [39].

How is histone synthesis increased? Two extreme possibilities may be identified:

(1) That the efficiency of histone synthesis is lower in the oocyte than the egg, i.e., every mRNA molecule is translated, but 20–100-times less efficiently in the oocyte;

(2) That the efficiency with which active histone mRNA is translated is the same in oocyte and egg, but that 20–100-times fewer mRNA molecules are translated; i.e., histone mRNA is stored, in the truest sense of the word.

An attempt to distinguish these alternatives was made by injecting constant amounts of embryonic sea urchin mRNA into oocytes and eggs and measuring its translation [40]. This approach has the serious drawback of using a heterologous mRNA which might not respond to *Xenopus* oocyte factors as would a *Xenopus* oocyte mRNA, but this risk must be taken if the product histones are to be distinguished from those made on endogenous templates. The result was that eggs were $\leqslant$1–2-times more efficient at translating injected histone mRNA than were oocytes. It was tentatively concluded that oocytes contain an untranslated pool of histone mRNA that is mobilised after maturation.

At the early stages histone synthesis is thus translationally controlled by some mechanism that distinguishes mRNAs encoding histones from other proteins, and H1 from the other histones. A simple calculation shows that post-transcriptional control is inevitable [40]. The rate of histone synthesis seen in the embryo requires at least 200 pg of mRNA. The oocyte contains only a 4C amount of DNA and 80–200 copies of the histone genes [51]. Thus, unless histone genes are amplified in the oocyte, ~40 days would be required to make this amount of mRNA, and the 2C zygote would require twice as long. The total time from oocyte maturation to the 30 000 cell stage is far less than a day, so de novo mRNA synthesis cannot make a significant contribution to histone synthesis till the histone gene concentration (i.e., number of nuclei) is sufficiently high. In principle, the 1000–2000 cell embryo contains sufficient genes to turn over the translated histone mRNA pool with a half-life of 3 h [40], assuming that all of the histone genes are active at this stage.

4. Histone mRNA stability

Since many weeks are needed to accumulate sufficient amounts of histone mRNAs, these mRNAs must be very stable in the oocyte. Presently available data indicates that some non-polyadenylated mRNAs are generally unstable in oocytes. Thus, injected depolyadenylated globin mRNA has a half-life of under 6 h in oocytes [42–44], in contrast to several weeks for polyadenylated globin mRNA [45]. In most cell types histone mRNA is mainly not polyadenylated [46–48], and as expected histone mRNA from HeLa cells [49] and sea urchin embryos [50] are functionally unstable in the oocyte. The synthetic activity of the sea urchin mRNA decays with a half-life of ~3 h. Why then is the endogenous histone mRNA of the oocyte stable? Part of the explanation may be that 50–75% of it is polyadenylated [51–54]. However 25–50% is not polyadenylated by the criterion that it fails to bind to oligo(dT) cellulose. It might have a short poly(A) tract, <20 residues long, but globin mRNA needs $\geqslant$20 adenylate residues to stabilise it [55]. Thus either:

(1) Oocyte non-polyadenylated mRNA is intrinsically more stable than that of sea urchins and HeLa; or

(2) It needs a shorter poly(A) tract than globin to stabilise it; or

(3) It is sequestered so that it is not available for degradation or translation.

In support of (1) it can be said that polyadenylation is not the only way of stabilising mRNAs at their 3′-end. Thus reexamination of the stability of naturally non-polyadenylated reovirus mRNAs injected into oocytes indicates that they are very stable, having a half-life of 3–5 days [56], rather more than the 18 h originally reported [57]. Interestingly, deadenylated mengovirus RNA is also highly stable in the oocyte [58], thus bringing further into question any simple physiological relationship between mRNA polyadenylation and stability.

Examination of the histone mRNA of eggs and embryos indicated that >90% of it is not polyadenylated [51,54], in contrast to 25–50% in oocytes. This switch is probably produced by deadenylation of oocyte polyadenylated mRNA [54]. Since the embryonic RNA is not polyadenylated it is likely, by analogy with injected sea urchin histone mRNA, that the whole pool of translated histone mRNA decays stochastically with a half-life of ~3 h. More direct evidence for this idea comes from experiments where the

instability of the endogenous H1 histone mRNA has been observed in androgenetic haploid hybrids of *Xenopus laevis* (♀) × *Xenopus borealis* (♂) [59]. In this hybrid the maternal chromosomes are destroyed by UV irradiation so that the cytoplasm, and hence the stored maternal mRNA is exclusively of the *laevis* type. Any newly synthesized mRNA must be exclusively programmed by *borealis* genes. Since H1 histones of the two species may be separated by electrophoresis, it is possible to follow the mobilisation and decay of the stored histone mRNA. As expected, the first H1 histone to be detected is maternal, i.e., made on stored transcripts; this is supplemented by *borealis* H1 made on new transcripts by the 1000–10 000 cell stage (see below). Soon after the gastrula stage maternal H1 synthesis ceases to be detectable, suggesting that the maternal mRNAs have been degraded. It is extremely difficult to obtain accurate kinetics in these experiments, but they are consistent with the 3 h half-life measured with sea urchin mRNA. It is curious that a specific mRNA held back for translation till the blastula stage should be lost only a few hours afterwards.

5. Transcription of the histone genes

The high histone mRNA content of the egg, together with the mRNA instability, means that the histone genes must be intensely active throughout oogenesis. For a long period after maturation new gene action can have little impact on histone synthesis, because the genes are so low in concentration. As pointed out in section 3, there are in principle sufficient genes to renew the pool of active histone mRNA in 3 h at the 1000–10 000 cell blastula stage. In the *X. laevis* × *X. borealis* hybrids new H1 gene action is detected at this stage and it has made a major quantitative contribution by the gastrula stage [59,60]. Actinomycin experiments indicating an earlier effect thus seem to be incorrect [16]. Presumably the non-H1 histone genes are also active by the blastula stage. It is not known if the H1 histone genes are inactive before the blastula stage because earlier there would have been too few genes to have had an observable effect.

6. Histone gene expression in other developing animals

The basic pattern of histone synthesis outlined above is likely to apply to other frogs and probably to amphibians in general. Shih et al. [61] have already shown this for *Rana pipiens*. There is, however, the surprising difference that H4, to a much greater extent than H1, is synthesised at low rates during cleavage. The deficiency is made good by the earlier synthesis of excess H4 during oogenesis.

Most other groups of animals begin development with a phase of rapid nuclear division, and therefore face similar problems to frogs. In *Drosophila* there is an initial phase of very rapid nuclear divisions in the absence of cell division, producing a syncytium. Just as in amphibians there is a pool of stored histone detectable in chromatin reconstruction experiments [62]. The mechanism of chromatin assembly may be somewhat different from that in *Xenopus*, in that the histone-binding, acidic protein [22] is absent. Instead there is another protein that first interacts with the DNA and then catalyses nucleosome assembly [62]. However more than one agent is likely to be involved in the intact embryo.

Coupling of DNA and histone synthesis was originally believed to occur in the sea urchin embryo, but recent reports show that histone synthesis is continuous during the cell cycle [63] and that there may be synthesis of small amounts of histone-like proteins in the oocyte [64,65]. The evidence is best for storage of H2B but is not yet conclusive [65]. Exact measurements of rates of histone synthesis in developing sea urchins have not yet been made, but it is quite certain that transcription of the histone genes is uncoupled from DNA synthesis, in that a maternal pool of histone mRNA is accumulated during oogenesis. However sea urchins differ from amphibians in that the genes make a significant contribution to histone synthesis from the very start of development [48,66,67]. This is achieved by a greater reiteration of histone genes than has yet been measured in any other phylum [42]. Another apparent difference is that the genes expressed in early development switch to a different type at the blastula or gastrula stage (reviewed in [48]). Such a phenomenon has not been detected in *Xenopus*, as judged by gel electrophoretic mobility although some rather weak evidence has been adduced to suggest a change in H1 [17]. An examination of mRNA sequences would be more informative, and has not yet been attempted.

One organism to which the amphibian pattern of histone synthesis might not apply is the mammal. The mammalian egg is small, divides at much the same

rate as a cultured cell and exists at 37°C. It could therefore regulate its histone synthesis like a somatic cell. However mammals had large-egged ancestors and might have a 'fossilized' version of protein synthesis regulation. It is already known that they have stored non-histone mRNAs [68].

7. Conclusions

The rapid assembly of new nuclei in early *Xenopus* embryos involves both the utilisation of a store of histone proteins and the mobilization of a pool of stored mRNA. The reason for using both processes is not clear, but evidently it works! The synthesis of H1 is translationally controlled separately from the other histones. The histone genes are very active by the 1000–10 000 cell stage, and perhaps before. By the gastrula stage all H1 histone, and perhaps the other histones too, is made on new transcripts. The stored maternal mRNA has almost disappeared by this stage, its decay probably being independent of the cell cycle. It is likely that the normal, cell cycle-dependent cells come into operation in the gastrula stage, but this remains to be established.

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