INTERNATIONAL

Review of Cytology

A SURVEY OF CELL BIOLOGY

VOLUME 105

INTERNATIONAL

REVIEW OF CYTOLOGY

A SURVEY OF CELL BIOLOGY

VOLUME 105

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 52-5203

ISBN 0–12–364505–0 (alk. paper)

PRINTED IN THE UNITED STATES OF AMERICA

86 87 88 89 9 8 7 6 5 4 3 2 1

Contents

Automated Individual Cell Analysis in Aquatic Research

CLARICE M. YENTSCH AND SHIRLEY A. POMPONI

Establishment of the Mechanism of Cytokinesis in Animal Cells

R. RAPPAPORT

The Circumventricular Organs of the Mammalian Brain with Special Reference to Monoaminergic Innervation

CLAUDE BOUCHAUD AND OLIVIER BOSLER

INTERNATIONAL REVIEW OF CYTOLOGY, VOL. 105

Remodeling of Nucleoproteins during Gametogenesis, Fertilization, and Early Development

DOMINIC POCCIA

Department of Biology, Amherst College, Amherst, Massachusetts 01002

I. Introduction

The chromosomes of virtually all eukaryotic cells consist minimally of DNA and histone proteins. However, it has long been known that the DNA-associated proteins of many mature sperm cells may be radically different from the histones characteristic of somatic cells (Miescher, 1897). In fact, the only known exceptions to the ubiquity of histones occur in male germ cell lineages, where histones are sometimes dispensed with altogether in postreplicative, posttranscriptive spermatids. In other cases, sperm-specific histone variants replace or supplement somatic-type histones. The near ubiquity of the histones and their unusually high degree of amino acid sequence conservation during evolution serve as evidence for a critical role in normal cellular physiology. Therefore, the unusual nucleoprotein composition of the mature sperm cell might be expected to be reversed soon after fertilization if the male chromatin is to behave properly in subsequent cell cycles.

Substitution of one or more histone variant subtypes by other variants or basic DNA-binding proteins represents a fundamental remodeling of the chromatin. Histone remodeling is likely to have major effects on the structure of the chromatin, since histone "function *is* structure, the proper dynamic packaging of DNA in the nucleus" (Simpson and Bergman, 1981).

In addition to the more extreme types of nucleoproteins or sperm-specific histone variants found in sperm cells, nonallelic variants of histones have been demonstrated in different somatic tissues and at different stages of embryonic development (Cohen *et al.*, 1975; Zweidler, 1984). Models have been devised for how chromatin composition might change in cell lineages in which different histone variants are synthesized at different times (Newrock *et al.*, 1978b; Weintraub *et al.*, 1978). Such replication-dependent remodeling differs from the major switching of histone or basic protein variants occurring in single-cell types without replication, for example, during spermatogenesis or pronuclear development (Dixon, 1972; Poccia *et al.*, 1984).

In this article, I will review what is known about the transformation of nu-

cleoprotein types during gametogenesis, fertilization, and early development, outline chromatin structural changes which accompany these nucleoprotein transitions, and speculate on some possible functions of the unique nucleoproteins and histone variants associated with gamete, zygote, and early embryonic nuclei.

II. Chromatin Structure and Histone Variation

The basic structure of the 10-nm-chromatin fiber is now well established and has been reviewed extensively (Elgin and Weintraub, 1975; Kornberg, 1977; Lilley and Pardon, 1979; Lewin, 1980; McGhee and Felsenfeld, 1980; Igo-Kemenes *et al.*, 1982; Weisbrod, 1982; Reeves, 1984). Two each of the four core histones (H2A, H2B, H3, and H4; molecular weights 10,000–16,000) form an octamer which protects 146 base pairs of DNA from micrococcal nuclease digestion. This DNA wraps about the octamer 1.75 turns to form the core nucleosome (Richmond *et al.*, 1984; Burlingame *et al.*, 1985). The central portion of the fifth histone, H1, is believed to bind to the DNA as it enters and exits the nucleosome to complete two full turns, conferring protection from micrococcal nuclease digestion on an additional 15–20 base pairs (bp) (Simpson, 1978; Allan *et al.*, 1980). The approximately 160-bp unit containing the core histone octamer and H1 has been termed the chromatosome (Simpson, 1978). H1 is also believed to associate with the variable amount of linker DNA which connects one nucleosome to the next along the chromatin fiber (Noll and Kornberg, 1977). The amount of linker DNA determines the average nucleosomal spacing or repeat length revealed by limited nuclease digestion. Linker DNA may also associate with N-terminal regions of core histones (Allan *et al.*, 1982). The 10-nm fiber organizes as a 30-nm fiber for which there are several models (Felsenfeld and McGhee, 1986). Above the level of the 30-nm fiber, little is known about the way in which the chromatin is packed. The most densely packed interphase chromatin is found in some sperm nuclei (Pogany *et al.*, 1981; Green and Poccia, 1985) and equals or exceeds in compaction metaphase chromosomes.

Histones are not as conserved as is often assumed. The most highly conserved histone, H4, which suffers just two conservative amino acid substitutions out of 102 residues between calf and pea (de Lange *et al.*, 1969), shows much greater variation (9–22%) in yeast, *Neurospora*, and *Tetrahymena* (Woudt *et al.*, 1983; Hayashi *et al.*, 1984; Glover and Gorovsky, 1979). In general, H3 is the next most conserved, H2B and H2A show greater variability, and H1 is the least conserved. This order also holds generally for variation within different tissues of a given organism. Since most organisms have multiple genes for histones, nonallelic variants are possible. For example, yeast has two genes coding for H2A subtypes different at 2 out of 131 amino acids (Choe *et al.*, 1982). Non-

allelic histone variants can be of the same size (homomorphic), differing in sequence, or of different sizes (heteromorphic) related by various insertions or deletions (West and Bonner, 1980).

Certain regions of histones are less conserved than others. In general, most differences are seen in the N-terminal portions of core histones, thus conserving the C-terminal regions which are involved in histone–histone interactions (Isenberg, 1979). In H1, variation is greatest on both sides of a more or less centrally located conserved segment (Allan *et al.*, 1980). Nonallelic homomorphic variants often differ in their hydrophobic regions and are usually best resolved using polyacrylamide gel electrophoresis in the presence of the nonionic detergent Triton X-100 (Zweidler, 1978). Protein sequencing and the sequencing of DNA clones continue to give more information on the range of histone variants found in nature. The list is far from complete for most organisms.

Although the synthesis of histones is often tightly linked to DNA synthesis, this is not always the case (Coffino *et al.*, 1984; Wu *et al.*, 1984). Zweidler (1984) has classified mammalian histone variants as replication dependent, partially replication dependent, replication independent, minor, and tissue specific. For example, the H1 variant H5 is erythrocyte specific; the H1o variant is associated with tissues that have ceased cell division. Such behavior suggests that different histone variants may serve different functions and that some might be restricted to subregions of the genome.

In addition to primary sequence variation, histones suffer various posttranslational modifications such as phosphorylation, acetylation, methylation, ubiquitination, and ADP-ribosylation, which can alter their charges, conformation, and strengths of binding to DNA. Most of these modifications take place in the N-terminal regions of core histones and most likely modulate the affinity of the histones for DNA (Isenberg, 1979). Secondary modifications may play a role in gene activation (Allfrey, 1977; Weisbrod, 1982).

III. Embryonic Histone Variants and Posttranslational Modifications

A. Histone Variants and Chromatin Remodeling in Early Development

Changes in H1 subtypes during early development have been reported for several organisms, but changes in core histones are apparently less common. However, the demonstration of core histone variants generally requires sensitive electrophoretic techniques which have not always been employed (Fig. 1). The best documented cases of histone remodeling in the early embryo are from the sea urchin. A list of references to known sequences of histones expressed in oocytes or early embryos is given in Table I.

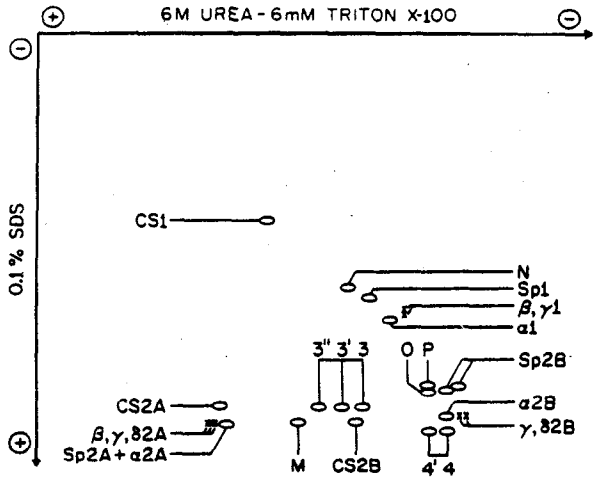


FIG. 1. Diagram of a two-dimensional gel electrophoretic separation of histones found in sea urchin development. Sp variants are found in mature sperm cells, CS proteins in oocytes and cleavage stages, and histones designated with Greek letters in embryos. Protein M is the H2A variant H2A.Z, N is phosphorylated Sp H1, and O and P are phosphorylated Sp H2Bs. From Poccia *et al.* (1981).

TABLE I

SEQUENCES FROM HISTONE GENES EXPRESSED IN OOCYTES OR EARLY EMBRYOS

Histone	P/D[a]	Reference
Chicken (*Gallus domesticus*)		
H1	D	Sugarman *et al.* (1983)
H2A	D	D'Andrea *et al.* (1981)
H2A.F	D	Harvey *et al.* (1983)
H2A/H2B	D	Harvey *et al.* (1982)
H2B	D	Grandy *et al.* (1982)
H3	D	Engel *et al.* (1982)
H4	D	Sugarman *et al.* (1983)
Newt (*Notophthalmus viridescens*)		
H1	D	Stephenson *et al.* (1981)
H2A	D	Stephenson *et al.* (1981)
H2B	D	Stephenson *et al.* (1981)
H3	D	Stephenson *et al.* (1981)
H4	D	Stephenson *et al.* (1981)

TABLE I (*Continued*)

Histone		P/D[a]	Reference
Frog (*Xenopus laevis*)			
H2A		D	Moorman *et al.* (1982)
H2B		D	Moorman *et al.* (1982)
H3		D	Moorman *et al.* (1980); Ruberti *et al.* (1982); Moorman *et al.* (1981)
H4		D	Turner and Woodland (1982)
H4		D	Turner and Woodland (1982); Moorman *et al.* (1981); Zernik *et al.* (1980)
Sea urchin			
H1	E[b] (*Strongylocentrotus purpuratus*)	D	Levy *et al.* (1982)
H2A	E (*S. purpuratus*)	D	Sures *et al.* (1976); Sures *et al.* (1978)
H2A	E (*Parechinus miliaris*)	D	Busslinger *et al.* (1980)
H2A	E (*P. miliaris*)	D	Schaffner *et al.* (1978)
H2A	E (*P. miliaris*)	D	Grosschedl *et al.* (1981); Birchmeier *et al.* (1982)
H2A	L[c] (*P. miliaris*)	D	Busslinger and Barberis (1985)
H2B1	E (*P. miliaris*)	D	Busslinger *et al.* (1980); Schaffner *et al.* (1978)
H2B2	E (*P. miliaris*)	D	Busslinger *et al.* (1980)
H2B	E (*S. purpuratus*)	D	Sures *et al.* (1978); Sures *et al.* (1976)
H2B	L (*P. miliaris*)	D	Busslinger and Barberis (1985)
H3	E (*S. purpuratus*)	D	Sures *et al.* (1978)
H3	E (*P. miliaris*)	D	Busslinger *et al.* (1980)
H3	L (*L. pictus*)	D	Childs *et al.* (1982)
H4	E (*S. purpuratus*)	D	Grunstein *et al.* (1981)
H4	L (*Lytechinus pictus*)	D	Childs *et al.* (1982)
H4	E (*P. miliaris*)	P	Wouters-Tyrou *et al.* (1976)
H4	E (*P. miliaris*)	D	Bussingler *et al.* (1980)
Wheat (*Triticum aestivum*)			
H2A(1)		P	Rodrigues *et al.* (1985)

[a] P, from protein sequence and D, from DNA sequence.
[b] Early.
[c] Late.

1. *H1 Histones*

a. *Sea Urchin.* The first report of modulations of H1 species during sea urchin (*Strongylocentrotus purpuratus*) development was by Hill *et al.* (1971). The predominant species at blastula was augmented by a species with faster mobility on acid–urea gels. It was not clear whether the H1s appearing in early and late embryogenesis were different variants or forms differing in phosphoryla-

tion state as found in *Arbacia lixula* (Ruiz-Carillo and Palau, 1973). It was later shown in *S. purpuratus* that the two H1 species were synthesized at different times (Seale and Aronson, 1973). Similar switches in H1 subtype were demonstrated for *Lytechinus pictus, A. punctulata* (Ruderman and Gross, 1974), and *Parenchinus angulosus* (Brandt *et al.*, 1979).

Labeled early blastula H1 is retained almost quantitatively in larval chromatin (Ruderman and Gross, 1974). After its synthesis ceases, the fraction of the total H1 complement contributed by early H1 decreases with the same kinetics as the fraction of total cell number (nuclear DNA) contributed by blastula to any given stage (Poccia and Hinegardner, 1975) which is consistent with a lack of turnover during development. Early H1 accumulates in embryonic cells which cease division early and therefore presumably make no late H1s to "dilute" the preexistent species (Pehrson and Cohen, 1985).

The switch in H1 subtypes between early and late stages was shown to be transcriptionally regulated by Arceci *et al.* (1976). In an *in vitro* cell-free translation system, RNA from unfertilized eggs codes for only early H1, but postgastrula RNA codes predominantly for late H1. These data also suggested that the H1 subtypes were not merely forms differing in secondary modifications but transcripts of different genes. This suggestion was confirmed by Newrock *et al.* (1978a) who showed that mRNAs extracted from polyribosomes from different embryonic stages in *S. purpuratus* code for three different H1 variants in an *in vitro* translation system in which secondary modifications were absent. The early H1 was called αH1 and the two later forms were named β and γ. The switch from early to late H1 is not affected by preventing cleavage (Brookbank, 1978), disrupting the cell cycle with hydroxyurea or polyspermy (Harrison and Wilt, 1982), nor by separating the 16-cell embryo into micro-, macro-, and mesomeres (Arceci and Gross, 1980a). Others, however, have claimed that histone synthesis is shut off in cells dissociated at the swimming blastula stage (di Liegro *et al.*, 1978). The H1 switch is apparently sensitive to the drug cordycepin (Brookbank, 1980).

Late H1 (postblastula) consists of at least two species (Ruderman and Gross, 1974; Poccia and Hinegardner, 1975; Gineitis *et al.*, 1976). Pehrson and Cohen (1984) report that the two late forms of H1 (β and γ) are retained in adult tissues, in addition to another H1 (λ) which has a low molecular weight and is not expressed before feeding larva. Sequence data are available for the embryonic and adult H1s of *P. angulosus* (Brandt *et al.*, 1979; de Groot *et al.*, 1983).

The H1 switches in *S. purpuratus* are not completely coordinate. Of the two electrophoretically resolved species of early H1 in *S. purpuratus*, the $H1\alpha_1$ ceases synthesis at about 400 cells (hatching blastula) and the $H1\alpha_2$ stops at about 700 cells (early gastrula) (Harrison and Wilt, 1982). Synthesis and incorporation into chromatin of the late H1s begin at about the 200–250 cell blastula (for H1γ) and the 250–300 cell stage (for H1β).

Senger *et al.* (1978) have reported that the early H1 of *A. punctulata* is made up of two variants whose synthetic patterns show a transient change at the 8-cell stage. An unusual H1-like molecule is synthesized even before the early H1s (Newrock *et al.*, 1978b). This species, called cleavage-stage (CS) H1, has solubility properties, staining characteristics, low Triton X-100 affinity, and amino acid composition which place it in the H1 class. It has a rather high molecular weight for an H1, originally estimated at 24,000–28,000, but probably closer to 34,000 (Newrock *et al.*, 1978b; Poccia, 1986). Cleavage-stage H1 reacts with H1-specific antibodies (Pehrson and Cohen, 1984). It is discussed further in Section V.

b. *Other Organisms.* Switches in H1 subtypes in the early embryos of other organisms are fairly common. In the echiuroid worm, *Urechis caupo*, two H1 variants have been detected (Das *et al.*, 1982; Franks and Davis, 1983). Germinal vesicles and cleavage-stage nuclei are enriched in the maternal H1m, whereas the embryonic form H1e becomes predominant in the later embryo. This shift is reflected in a shift of synthesis of H1 from oocytes to early embryos. The surf clam, *Spisula solidissima*, has RNA coding for two H1 subtypes whose synthesis switches at the 32–64 cell stage (Gabrielli and Baglioni, 1975, 1977). In the snail *Ilyanassa obsoleta*, several H1 subtypes were identified which show differential synthetic patterns during development (Mackay and Newrock, 1982).

Most workers have reported no synthesis or change in the set of H1 histone variants of the frog *Xenopus laevis* in early development (Destrée *et al.*, 1973; Byrd and Kasinsky, 1973a,b; Adamson and Woodland, 1974; Cassidy and Blackler, 1978; Flynn and Woodland, 1980). Others have claimed that a shift in H1 subtypes detectable on Triton gels is seen in a comparison of histones labeled from the 8-cell stage to blastula and late blastula to neurula (Köster *et al.*, 1979). However, these shifts may result from differences in secondary modifications (van Dongen *et al.*, 1983). Several H1 variants were found in later embryos and adult tissues and two were apparently adult specific, possibly related to H5 or H1o (Risley and Eckhardt, 1981; Moorman and de Beer, 1985). Genes for several different *Xenopus* H1 variants, which exist in different arrangements, have been isolated (Destrée *et al.*, 1984). One gene cluster is expressed in oocytes, gastrula stage, and erythroblasts. An H1o/H5-like variant has been detected cytochemically in many adult tissues of *Xenopus*, but not in oocyte or mature sperm nuclei (Moorman and de Boer, 1985). It was, however, present in spermatogenic cells.

2. *Core Histones*

a. *Sea Urchin.* An elaborate developmentally regulated program of core histone variant incorporation into chromatin takes place in early sea urchin embryos. The urchin has been the most intensively studied and may be the

organism which possesses the most extreme diversity of histones. For example, at least 24 histone variants are known in *P. angulosus*, not counting CS subtypes (Schwager *et al.*, 1983; Brandt *et al.*, 1979).

It is worth reviewing the progress made in identifying histone subtypes in the sea urchin since few other organisms have received the kind of scrutiny that it has.. Early experiments were hampered by the inadequacy of electrophoretic systems used and contamination problems, particularly in early stages. For example, Orengo and Hnilica (1970) reported typical histones in hatching blastula and gastrula stages, but unusual arginine-rich proteins in the 4- to 8-cell stage. Johnson and Hnilica (1970) could not find typical histones in the chromatin before the 64-cell stage, although they reported histone synthesis and therefore suggested a lag in the incorporation of the histones into nuclei. Benttinen and Comb (1971) found nonstoichiometric ratios of core histones. Crane and Villee (1971) and Thaler *et al.* (1970) compared gastrula, sperm, and unfertilized egg histones, but inadequate resolution and contamination problems make interpretation of these patterns difficult.

Changes in core histone patterns between blastula and pluteus could not be distinguished by Marushige and Ozaki (1967). Vorob'yev *et al.* (1969) suggested that there were quantitative differences in the arginine-rich histones between blastula and gastrula. Clear histone patterns on high-resolution gels were obtained by Hill *et al.* (1971). These gels revealed multiple-core species with one H2A increasing and one H3 decreasing from blastula to pluteus. Whether these were differences in modified forms or in primary structure could not be demonstrated. Seale and Aronson (1973) found no differences in core histones between the 16-cell stage and pluteus and found only H2A and H2B before this stage. Ruiz-Carillo and Palau (1973) found quantitative differences in core histone fractions between blastula and gastrula and heterogeneity in the H3 and H4 fractions due to acetylation. Poccia and Hinegardner (1975) found differences in the H2A and H2B fractions, with the apparent loss of two H2B species by late larval stages. Similar results were reported by Gineitis *et al.* (1976) who also showed that the patterns remained the same in animalized, vegetalized, or normal embryos.

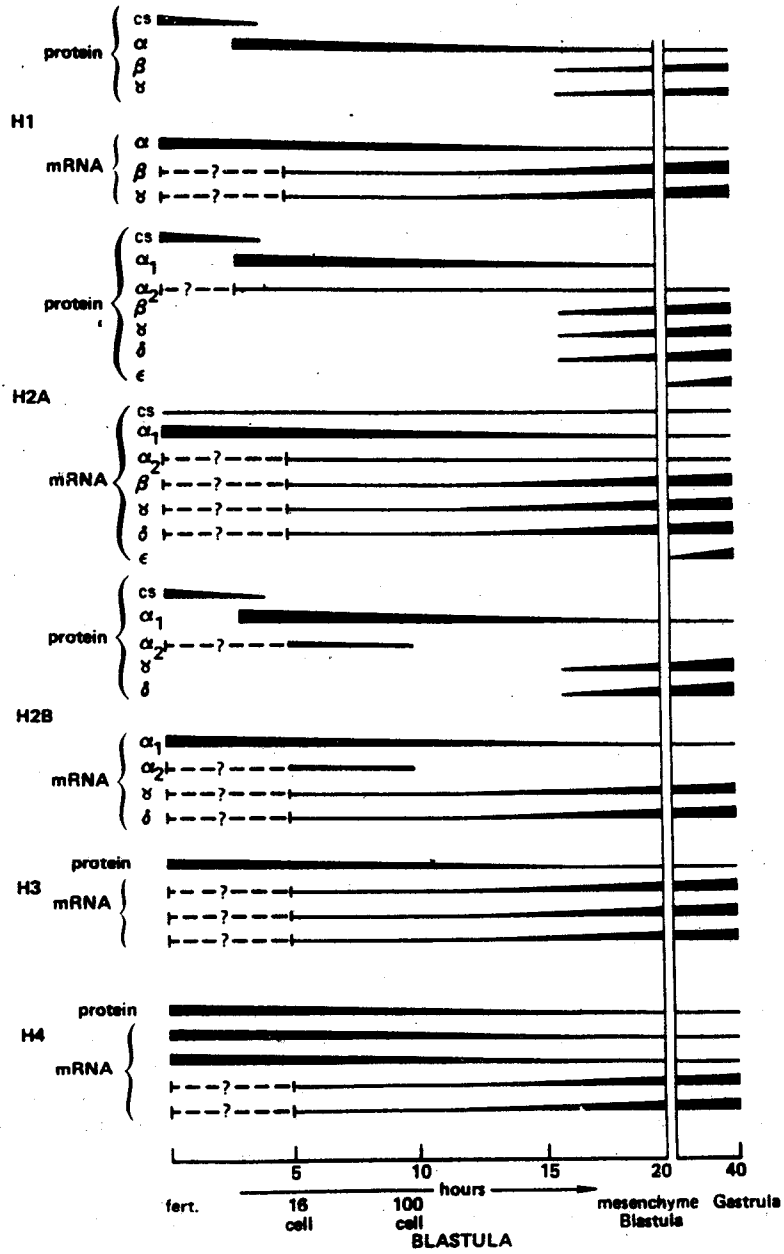
Much of the confusion regarding the designation of histone bands on gels due to primary structural variation or secondary modification was eliminated only with the introduction of gel systems of greater resolution (Zweidler and Cohen, 1972; Cohen et al., 1973; Savić and Poccia, 1978), of *in vitro* translation systems (Arceci *et al.*, 1976; Newrock *et al.*, 1978a), and of protein sequencing (von Holt *et al.*, 1984). By pulse-labeling histones of *S. purpuratus* with [^{3}H]leucine and the analysis of acid-extracted chromatin at various stages on gels containing the nonionic detergent Triton X-100, Cohen *et al.* (1975) demonstrated convincingly that a set of stage-specific switches in core histone variant synthesis occurred in early sea urchin development. Histones incorporated into early chro-

matin did not turn over extensively and late forms were not derived from early forms. They suggested that these forms differed in primary structure. This work was extended by Newrock *et al.* (1978b) who identified additional components. Similar switches of core variants have been demonstrated in the chromatin of other sea urchin species (Treigyte and Gineitis, 1979; Brandt *et al.*, 1979; von Holt *et al.*, 1984)

The known core variants are in the H2A and H2B classes (Figs. 1 and 2); all sequenced sea urchin genes or proteins of H3 or H4 are identical within each class (Childs *et al.*, 1982). The first synthesized histone variants are CS histones (Newrock *et al.*, 1978b). These are synthesized between fertilization and morula, after which their synthesis ceases. A second set of variants (α) is synthesized by at least the third S phase after fertilization until the blastula stage. Another set of variants (β, γ, ϵ) begins to be made during blastula with the late forms of H2A and H2B synthesized slightly later than the late forms of H1 (Harrison and Wilt, 1982). As a consequence, the composition of the chromatin changes throughout the cell cycles of early development as new histone variants are incorporated, while the preceding variants are, for the most part, retained (Ruderman and Gross, 1974; Poccia and Hinegardner, 1975; Cohen *et al.*, 1975; Newrock *et al.*, 1978b; Arceci and Gross, 1980b).

That the histone variants in early sea urchin development actually differ in primary structure was proved by *in vitro* translation of mRNA from different stages of development (Newrock *et al.*, 1978a; Weinberg *et al.*, 1977; Hieter *et al.*, 1979; Childs *et al.*, 1979). Newrock *et al.* (1978a) showed that *in vitro* translation of morula RNA produces only α variants, blastula polysomal RNA gives almost entirely α variants, but gastrula stage RNA codes predominantly for later types. Typical modified forms due to acetylation are not made in the *in vitro* system. No late forms were seen in the translation of mRNA from the total RNA of early embryos.

Childs *et al.* (1979) showed that the transitions of mRNAs in the early to late histone switches are not abrupt, since small amounts of late mRNAs could be detected in early stages and small amounts of early mRNAs were synthesized during gastrula. An additional early (α) and a late (ϵ) H2A variant were found, and CS H2A mRNA assayed by cell-free translation was found in late stages as well as in unfertilized whole egg RNA (Fig. 2). Low levels of late histone mRNAs can be detected in oocytes (Knowles and Childs, 1984; Busslinger and Barberis, 1985). Spinelli *et al.* (1979) confirmed the shift from early to late mRNAs in *Parechinus lividus* but did not detect any CS variant mRNAs. However, they selected newly synthesized histone mRNAs with a recombinant probe containing α histone genes, so CS mRNAs might have escaped detection. They suggested that either CS transcripts were present in very low concentrations, were subtypes of α mRNAs, or were synthesized in early but not mature oocytes. Shifts in mRNA populations from early to late stages are also seen for


protein
cs
α
β
γ
H1
mRNA
α
β
γ
protein
cs
α1
α2
β
γ
δ
ε
H2A
mRNA
cs
α1
α2
β
γ
δ
ε
protein
cs
α1
α2
γ
δ
H2B
mRNA
α1
α2
γ
δ
protein
H3
mRNA
protein
H4
mRNA
5
10
15
20
40
hours
fert.
16 cell
100 cell
BLASTULA
mesenchyme Blastula
Gastrula

histone genes that code for identical proteins such as H4s (Grunstein, 1978). *In situ* hybridization experiments have shown that the α mRNAs are uniformly distributed in cleaving embryos, whereas, at blastula, cells in certain regions become depleted (Cox *et al.* 1984). The shift from early to late histone mRNAs is not regulated differently in various cell blastomeres and subsequent differences in the ratio of early to late mRNAs may be a simple reflection of variation in cell-cycle progression for different lineages (Angerer *et al.*, 1985).

To date, early and late sea urchin histone variant genes have been isolated but CS genes have not (Kedes and Birnstiel, 1971; Overton and Weinberg, 1978; Maxson *et al.*, 1983b; Childs et al., 1982; Busslinger and Barberis, 1985). Most of our knowledge of embryonic histone amino acid sequences is derived from these isolated genes. The expression of sea urchin histone genes has been extensively reviewed elsewhere (Kedes, 1976, 1979; Hentschel and Birnstiel, 1981; Weinberg *et al.*, 1983; Maxson *et al.*, 1983a,b).

The α and later variants are similar in size and sequence (see Table I for references; also von Holt *et al.*, 1979, and Schwager *et al.*, 1983, for partial sequences including H2B from the adult intestine). The most variable regions occur at the ends of the molecules. Within the H3 or H4 classes, early and late genes code for identical proteins and the sequences determined for different species are also identical (Childs *et al.*, 1982).

The CS proteins differ most radically on gels from the later embryo histones of their classes. CS H2A has the high Triton affinity characteristic of H2As, is larger than somatic or embryonic H2As (heteromorphic), and reacts with an H2A-specific antibody that recognizes α and all later H2As except an H2A variant called Y6, Z, or M (Newrock *et al.*, 1982; Newrock *et al.*, 1978b; Wu *et al.*, 1982; Poccia *et al.*, 1981). CS H2B is essentially the same size as somatic or embryonic H2Bs (homomorphic) but has a higher Triton affinity. CS H2A and CS H2B behave as expected for core nucleosomal proteins upon digestion of chromatin with micrococcal nuclease (Shaw *et al.*, 1981) and during replication (Poccia *et al.*, 1981, 1984). The designation CS is somewhat of a misnomer, since CS proteins are synthesized in oocytes (Herlands *et al.*, 1982). CS histones accumulate in a storage pool in sea urchin eggs (see Section V,B). The pool does not contain α or later subtypes (Poccia *et al.*, 1981; Salik *et al.*, 1981).

In addition to the rather extensive switching of histone variants in sea urchins, most of the histones are actively modified posttranslationally during develop-

FIG. 2. Developmental expression of sea urchin histone genes. The pattern of expression of *S. purpuratus* histone genes in early development is indicated by thick lines for abundant components and thin lines for relatively minor components. Broken lines indicate uncertainty about the synthesis of a species. Data are based on *in vivo* protein synthesis, *in vitro* cell-free translation, or the pulse-labeled *in vivo* mRNA experiments of Newrock *et al.* (1977) and Childs *et al.* (1979). From Childs *et al.* (1979).

ment. Although mature sperm (Sp) variants show no microheterogeneity due to secondary modifications (Easton and Chalkley, 1972), Sp H1 and Sp H2B become phosphorylated after fertilization (Green and Poccia, 1985). Acetylation accounts for most of the heterogeneity of embryonic H3 and H4 fractions (Burdick and Taylor, 1976; Treigyte and Gineitis, 1979). In an *in vitro* system, Horiuchi *et al.* (1984) found that the rates of histone acetylation and deacetylation in isolated sea urchin nuclei remained at a high and constant level between morula and gastrula stages. Chambers and Shaw (1984), however, found that the amount of diacetylated H4 declined with development and suggested a correlation with the decreasing rate of cell division seen in the early embryo. H2A and H2B, but not H1 variants, also seem to be acetylated (Horiuchi *et al.*, 1984; Chambers and Shaw, 1984) but these were not directly investigated.

b. *Other Organisms.* Switches in core histone subtypes analogous to the sea urchin switches have been found in a limited number of organisms. During development of the mud snail, *I. obsoleta*, there appears to be an H2A and an H2B component which are synthesized in oogenesis but not during early embryogenesis, an H2A and H2B synthesized only during early embryogenesis, and an additional set of H2Bs which corresponds to sea urchin late histones appearing in the veliger larva stage (Mackay and Newrock, 1982).

In the chicken, new H2B and H3 variants appear during somite formation (Urban and Zweidler, 1983). All variants present at this stage continue to be expressed in the adult. Most chicken histone genes isolated so far are expressed in the embryo but not in the adult chicken (Engel, 1984). These might be specific to embryonic cell types or to cells undergoing rapid proliferation. The chicken variant H2A.F appears to be activated relative to the major H2A variant (H2A.1) during early development and expressed in some but not all adult tissue. It is 40% divergent from H2A.1 (Harvey *et al.*, 1983). Engel (1984) suggests that because of sequence divergence, not all chicken genes may have been accounted for in searches using embryonic or heterologous probes.

Wheat embryos contain at least three histone H2A variants, one of which has been completely sequenced (Rodrigues *et al.*, 1985). This one is unusual in that it has a 19 amino acid C-terminal extension which has some sequence homology with H1s.

In some organisms, no major changes are detected in the core histone complement in early development. Imoh (1978) could find no differences on Triton gels in histones of the newt *Triturus pyrrhogaster* from blastula to tail bud. No one has found core histone variants in *X. laevis* (Woodland, 1980, 1982). Although it is not certain whether some special variants are synthesized in the first several cell cycles in this large egg, it is likely that *Xenopus* possesses limited heterogeneity within its histone classes. Hybrid selected mRNAs from ovary, blastula, or neurula, when translated in a cell-free system, all code for the same set of