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Developmental Regulation of Two 5*S* Ribosomal RNA Genes

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The developmental regulation of two kinds of *Xenopus* 5*S* RNA genes (oocyte and somatic types) can be explained by differences in the stability of protein-protein and protein-DNA interactions in a transcription complex that directs transcription initiation by RNA polymerase III. Dissociation of transcription factors from oocyte 5*S* RNA genes during development allows them to be repressed by chromatin assembly. In the same cells, somatic 5*S* RNA genes remain active because their transcription complexes are stable.

THE FROG *XENOPUS LAEVIS* CONTAINS TWO KINDS OF multigene families that encode 5*S* ribosomal RNA, an essential component of ribosomes. *Xenopus* oocytes synthesize and accumulate large amounts of 5*S* RNA encoded for by a large multigene family called oocyte 5*S* DNA. Oocytes also express a small multigene family (somatic 5*S* DNA). After fertilization and development of the embryo, the oocyte-specific 5*S* RNA genes are repressed, while the somatic 5*S* RNA genes remain active. This is an example of what may be a common developmental mechanism: where two (or more) gene families have similar (but not identical) cis-acting controlling elements that are recognized by the same factors but are nonetheless controlled differently. In studying the developmental control of this "dual" 5*S* RNA gene system, we have sought to understand the molecular mechanisms that establish and maintain this pattern of differential gene activity.

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The 5*S* RNA Genes of *Xenopus laevis*

The structure and organization of the three kinds of 5*S* RNA genes that have been characterized from the *X. laevis* genome are diagrammed in Fig. 1. These are called major oocyte (*Xlo*) (*1*), trace oocyte (*Xlt*), and somatic (*Xls*) 5*S* DNA (*2*). Each class is organized in clusters of simple tandem repeats. All three classes are transcribed in growing oocytes, while somatic 5*S* DNA transcription contributes more than 95% of the 5*S* RNA synthesized in somatic cells (*3*). Because there are only 400 somatic 5*S* RNA genes but over 20,000 oocyte 5*S* RNA genes per haploid genome, this is a final differential gene transcription of over 1000-fold in somatic cells. We refer to this as a somatic-to-oocyte ratio (S/O) of 1000. The two kinds of oocyte-specific 5*S* RNA genes (*Xlt* and *Xlo*) are similar enough that we will concentrate on the differential transcription of *Xlo* and *Xls* 5*S* RNA genes in this article. There are six nucleotide differences between *Xlo* and *Xls* 5*S* RNA genes (Fig. 1), and the spacers are completely different except for short conserved elements near the 5′ and 3′ ends of the gene. We assess the importance of these sequence differences by in vitro (*4*) and in vivo (*5*) transcription assays, where full-length 5*S* RNA is synthesized.

The 5*S* RNA genes are accurately and efficiently transcribed by RNA polymerase III when they are injected into oocyte nuclei or incubated in extracts of these same nuclei. Transcription initiation is

influenced by a sequence 5′ to the gene and by an intragenic promoter element called the internal control region (ICR) (*6*). The ICR is the binding site for a 38,000-dalton protein called TFIIIA (*7*). This protein contains zinc (*8*) and is composed predominantly of nine imperfect tandemly repeated regions, each approximately 30 amino acids in length (*9*). Each repeated sequence, called a "zinc finger," includes pairs of cysteines and histidines that bind one zinc ion and constitutes a distinct module in the protein (*10*). Experiments with proteolytic fragments (*11*) and deletion mutants (*12*) of TFIIIA support the modular nature of TFIIIA that is predicted by the zinc finger model. One molecule of TFIIIA binds to the ICR through its nine zinc fingers; its carboxyl terminus is oriented toward the 5′ end of the 5*S* RNA gene and its amino terminus toward the 3′ end of the gene.

Transcription factor TFIIIA along with at least two other factors (TFIIIB and TFIIIC) form a transcription complex on the ICR that directs RNA polymerase III to initiate transcription (*13*). The ICR is required and sufficient to form a stable transcription complex in certain extracts in vitro; the upstream sequence is involved in directing polymerase to initiate accurately at the first nucleotide of the gene (*6*). Polymerase III transcribes the 5*S* RNA gene without dislodging the transcription complex (*14*) and terminates transcription at a consensus sequence (*15*). Purified RNA polymerase III alone recognizes this termination sequence (*16*), although other factors may be involved in facilitating termination (*17*).

The Active State of a 5*S* RNA Gene: The Transcription Complex

Evidence to date suggests that multiple different proteins combine to form preinitiation or transcription complexes on promoter sequences for eukaryotic genes transcribed by all three forms of RNA polymerase (*14, 18*). These interactions have been proposed to occur over considerable distances adjacent to and in some cases within genes transcribed by RNA polymerases II (*19*). The advantages of multiple protein-DNA interactions lie in increasing the specificity and stability of the nucleoprotein complex. If several different proteins interacted with the same DNA sequence, then small changes in this sequence (such as occur between the oocyte and somatic 5*S* RNA genes) that altered subtly the binding constant of each protein could greatly change the binding of the entire complex.

The binding of the transcription factor TFIIIA alone to a 5*S* RNA gene is relatively weak [$K_d = 10^{-9}$ (*20*)]. The affinity of TFIIIA for a 5*S* RNA gene is increased by interaction with a second transcription factor, TFIIIC, in a rapid reaction (*14*) (Fig. 2). The transcription factors TFIIIA and TFIIIC in combination bind so tightly to a somatic 5*S* RNA gene that they do not dissociate if free factor molecules are removed from solution (*21*) or if a large excess of competitor 5*S* RNA is used to challenge the complex (*14, 22*). Stabilization is not sufficient for transcription since the TFIIIA/TFIIIC/5*S* DNA complex requires yet another transcription factor to be present before the complex can be recognized by RNA polymerase III. As we discuss later, transcription of 5*S* RNA genes can occur under conditions where the complex is not stable (*23*). Therefore, stabilization of a transcription complex is neither required nor sufficient for transcription initiation. The importance of transcription complex stability lies in its role in the control of gene expression. Although the molecular details of the stabilization process are unknown, possible mechanisms include a conformational change in TFIIIA structure, a closed to open complex transition analogous to RNA polymerase initiation in prokaryotes (*24*), a cooperative interaction between TFIIIA and TFIIIC, or a covalent modification of TFIIIA, for example, a dephosphorylation or phosphorylation catalyzed by TFIIIC.

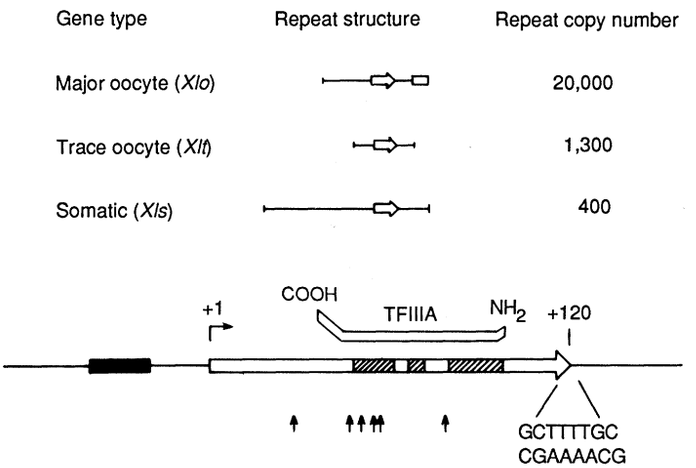

Fig. 1. The 5*S* RNA genes of *Xenopus laevis*. Each class is organized in clusters of simple tandem repeats. One repeating unit of each is drawn to scale. The major oocyte gene family (*Xlo*) is located mainly at the telomeres of most chromosomes in clusters of over 1000 repeats (*75*). Each repeating unit of 650 to 850 bp consists of a gene and a pseudogene separated by an AT-rich spacer (*1*). At least one major cluster of repeats of the trace oocyte family has been localized at the distal end of chromosome 13 (*76*). There are 1300 copies of *Xlt*, each 310 bp in length. The *Xlt* spacer region is again AT-rich (*2*). Each repeating unit of the somatic family (*Xls*) is 880 bp in length (*2*). There are approximately 400 copies of *Xls* most of which are in a single cluster on one chromosome (*76*). The intergenic spacers of *Xls* are very GC-rich (*2*). A typical 5*S* RNA gene is shown including sequence features of defined significance. A region just 5′ to the gene (solid rectangle) is required for accurate initiation of RNA polymerase (*6*), the region inside the gene constitutes the binding sites of TFIIIA and TFIIIC within the ICR. A consensus termination signal at the end of the 5*S* RNA gene (+120) is also shown (*15*). Vertical arrows indicate the six sequence differences (single base pair) between major oocyte and somatic 5*S* RNA.

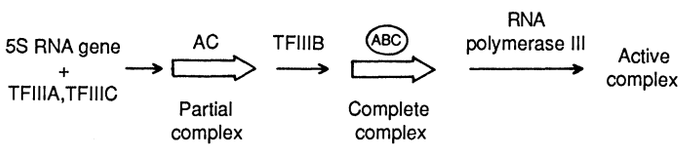

Fig. 2. The active state, a transcription complex. TFIIIA and TFIIIC bind rapidly to a 5*S* RNA gene nucleating a TFIIIA/TFIIIC/5*S* DNA complex. The TFIIIA/TFIIIC/5*S* DNA complex matures as TFIIIB is sequestered, forming a complete transcription complex. The complex is then competent to bind RNA polymerase III and transcription initiates.

A third activity, called TFIIIB, is required to form a competent transcription complex (*13*). TFIIIB is not a DNA binding protein (*21*) but acts on a TFIIIA/TFIIIC/5*S* DNA complex in a time-dependent manner, accounting for a lag period before synthesis of 5*S* RNA reaches maximal rates (*21, 25*). Although both TFIIIB and TFIIIC appear to be consumed by transcription complex formation (*21, 22*), their actual inclusion in a complex has not been proved. Since TFIIIB and TFIIIC have not been purified, it remains possible that the crude fractions contain multiple activities required for complementing transcription in vitro (*26*). Factors included in the TFIIIB and TFIIIC fractions are required for the transcription of other class III genes; TFIIIA is the only factor identified to date that is specific for 5*S* DNA transcription. The evidence that TFIIIA is included in a transcription complex comes from deoxyribonuclease (DNase) I footprinting of the complete transcription complex (*27*), which shows an unmistakable protection pattern characteristic of TFIIIA binding to the ICR, although some slight modifications are apparent.

RNA polymerase III is not a part of the transcription complex (*21*). This large and complex enzyme recognizes and binds to transcription complexes. Herein lies one of the features of eukaryotic transcription that contributes to the specificity and logistics of transcription (*28*). Each of the three forms of RNA polymerase recognizes only its cognate transcription complexes. Since RNA polymerases also bind to naked DNA, the suppression of nonspecific binding sites for polymerases is one of the roles played by the assembly of inactive genes into chromatin (*29*). This renders the majority of DNA in a nucleus invisible to the polymerase as well as to free transcription factors in the nucleus (see later). This invisibility greatly increases the signal-to-noise ratio for an RNA polymerase molecule in its search for its cognate transcription complex (*30*).

The Repressed State: Chromatin Assembly

In somatic cells of *Xenopus*, the somatic 5*S* RNA genes are active (in stable transcription complexes) and the oocyte 5*S* RNA genes are repressed. This developmental state can be studied in vitro because active RNA polymerase III genes retain their ability to initiate transcription when isolated chromatin is used as a template (*14, 31*).

Oocyte 5*S* RNA genes remain stably repressed in chromatin isolated from somatic cells (*14, 32*). Even the addition of all factors (TFIIIA, and fractions containing TFIIIB and TFIIIC) plus RNA polymerase III to the chromatin template does not activate the oocyte 5*S* RNA genes. Oocyte 5*S* RNA genes in somatic chromatin can be activated by added transcription factors, but only after histone H1 is removed from the chromatin. Even short fragments of chromatin that cannot compact into higher order structures (solenoids) (*33*) can maintain their oocyte 5*S* RNA genes stably repressed (*32, 34*). Readdition of histone H1 to chromatin that has been previously depleted of the protein will restore the repressed state at levels of one molecule of histone H1 per nucleosome (*32*). Under these conditions of reassociation, histone H1 is believed to reconstitute a native chromatin confirmation (*35*). Therefore, oocyte 5*S* RNA genes in somatic cells are not associated with transcription factors but instead are complexed with nucleosomes and histone H1. We have no information on the location or abundance of nucleosomes near the active somatic 5*S* RNA genes.

Although the presence of histone H1 on nucleosomes is adequate to maintain repression in extracts in vitro, the stability of the repressed state of chromatin in vivo is likely to vary depending upon the extent of compaction of the chromatin (*36*) (Fig. 3) and the availability of histone H1 for exchange from the chromatin (*37*). One period of the cell cycle when chromatin structure must be disrupted is the S phase at the instant of DNA replication. The reassembly of chromatin after replication is not instantaneous; it involves a step-by-step maturation. First, the components of the histone octamer are added to newly replicated DNA forming nucleosomes (*38*). When these are in place, histone H1 binds to each nucleosome presumably at the entrance and exit of DNA that encircles the histone octamer (*33, 39*). With histone H1 in place, higher order structures are presumed to be able to form by virtue of cooperative interactions between histone H1 molecules on neighboring nucleosomes (Fig. 3).

If activation of a gene requires removal (or exchange) of histone H1, then the period just after DNA replication, when chromatin is immature, is a moment when controlling sequences in the DNA are especially accessible to DNA binding proteins. Additionally, any change in the binding constant of histone H1 to a nucleosome may influence the rate of histone H1 exchange and therefore the ease with which the underlying promoter can be programmed with

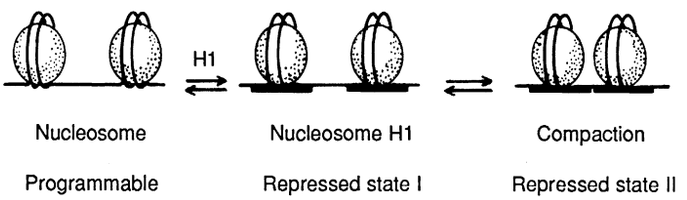

Fig. 3. The repressed state. A nucleosome consisting of DNA wrapped around an octamer of histone proteins does not inhibit the binding of transcription factors to the promoter (ICR) of a 5*S* RNA gene. However, when histone H1 (the solid bar) binds to nucleosomes and to part of the linker DNA between nucleosomes (*77*), the underlying DNA in this structure cannot be programmed into a transcription complex. The nucleosomes containing histone H1 interact, compacting the chromatin. This state of chromatin can occur when there are more than six nucleosomes with H1 in a row (*33*) and is considered to be a more inaccessible or repressed structure, because histone H1 is much less likely to exchange. Maturation of chromatin after DNA replication proceeds from left to right.

transcription factors. Such changes could be due to the presence of a particular tight binding subtype or covalent modification of histone H1 or of the core histones themselves. The most inaccessible DNA will certainly be that which has become most compacted into solenoid structures, and the DNA that most readily compacts into solenoids will be long stretches of regular nucleosomes that are not interrupted by other protein complexes such as transcription complexes.

The terms "open" and "closed" chromatin have often been applied to the structure of chromatin that surrounds active and repressed genes, respectively (*40*). The difference between these chromatin states experimentally is assessed by their inaccessibility to enzymes such as DNase I that more efficiently digest the DNA in "open" than "closed" chromatin. "Open" chromatin can include and surround entire transcription units; this may reflect the inhibition of chromatin compaction caused when transcription complexes interrupt regular nucleosome formation. Chromatin that is not compacted can more readily exchange its histone H1.

Maintenance of Stable Inactive and Active States of Gene Expression

The presence of stable transcription complexes on somatic 5*S* RNA genes and the repressed chromatin structure of oocyte 5*S* RNA genes represent the end state of the differential expression of these two classes of 5*S* RNA genes that is established by the end of gastrulation during embryogenesis. Even in cells that no longer synthesize 5*S* RNA, such as a nucleated *Xenopus* erythrocyte, a cell that has no detectable RNA polymerase III (*41*) and no detectable free TFIIIA (*42*), somatic 5*S* RNA genes remain in stable transcription complexes for days and probably for weeks.

We proposed that these two molecular states help account for the well-known biological phenomenon called maintenance of the differentiated state (*14, 28*). This refers to the fact that in nondividing, terminally differentiated cells the same genes remain active and the same genes remain repressed for long periods of time. Stable transcription complexes formed by the interaction of multiple transcription factors with their cognate DNA sequences maintain genes in an active state. This state of transcriptional competence is resistant to the repression caused by chromatin assembly. Stable repression is imposed by chromatin assembly on genes that lack transcription complexes. We propose that stable repression is imposed by regularly spaced nucleosomes that can interact with histone H1 and compact in such a way that the repressed gene becomes inaccessible to free transcription factors.

Transcription complexes on somatic 5S RNA genes have been shown to withstand many rounds of transcription without being released from the gene (*14, 28*). The same has been shown for histone octamers, which are not displaced by the progression of RNA polymerase during RNA synthesis (*43*). Presumably histone H1 must be displaced from a nucleosome for RNA transcription to pass it, although this fact has not been demonstrated. These two kinds of nucleoprotein structures (active transcription complexes and nucleosomes) have been designed to remain in place while permitting the passage of RNA polymerase. We predict that this will be a fundamental characteristic of any DNA-protein complex in the nucleus of a terminally differentiated cell that sits in the path of RNA polymerase, for example, proteins bound to enhancer elements located within introns of genes. If we may speculate further, protein-enhancer complexes might be located in introns just so that they can inhibit the compaction of an active gene into higher order structures by interrupting the regular array of nucleosomes along the transcription unit.

The Effect of Cell Division (DNA Replication) on Gene Expression

Cell division (used in this discussion interchangeably with "DNA replication") is said to be required for the change in state that occurs in some developmental systems (*44*). A characteristic feature of many cell lines in culture and cells in vivo is that they do not express certain genes until the cells stop dividing and are terminally differentiated. These observations suggest that DNA replication must influence gene expression. It does not say that changes in gene expression require DNA replication (*45, 46*).

An extremely important kind of biological memory is cell commitment or cell determination through which cells retain their phenotype during many rounds of division. A transcription complex could play a role in such a memory if one or more of its components remained in place long enough after passage of a replication fork to nucleate the formation of a new transcription complex (*28*). This possibility was tested directly by replication through a transcription complex in vitro, to determine whether such a memory could be transmitted to daughter chromatids (*47*). The result showed clearly that the preexisting complex was erased by passage of the replication fork, and there was no memory imposed by a preexisting complex. For this discussion, we generalize this observation, and conclude that the same transcription complexes that can withstand passage of RNA polymerase are erased at each round of DNA replication.

If this is true, then replication disrupts both the active and the repressed state of a gene providing a brief interval, while newly replicated chromatin matures, for the state of gene expression to be influenced toward activity or repression (see Fig. 3) (*38, 48*). The outcome of this perturbation for any gene depends upon the result of a competition between the formation of active stable complexes and the inexorable formation of mature nucleosomes complexed with histone H1 and compaction. In somatic cells, a somatic 5S RNA gene forms a stable complex rapidly even at low levels of transcription factors. This stable complex not only activates the somatic 5S RNA gene, but its presence withstands the competitive process of nucleosome assembly. In contrast, the oocyte 5S RNA genes can only form unstable complexes that require continued high levels of factors to maintain activity (*23*). Once unprogrammed oocyte 5S RNA genes become wrapped into chromatin, even high levels of factors cannot activate the genes (*49*).

If daughter genes are most susceptible to reprogramming just at S phase, then that is the time when free factors will have the greatest influence on gene activity. If a transcription complex is sufficiently stable, such as that formed on a somatic 5S RNA gene, then the continued activity of that gene is assured until the next round of DNA replication. If the timing of the synthesis and degradation of a limiting transcription factor is coupled precisely with one part of the S phase, then even two identical genes can be differentially controlled if they are replicated at different times in the S phase. This is the case for genes on the active and inactive X-chromosomes of female mammals (*50*). The differential expression of identical genes may be a consequence of the early replication of active genes in S phase and late replication of inactive genes. These ideas focus attention on the importance of cell cycle–regulated events that influence gene expression. There are a number of genes that are activated at S phase; the best known are genes for histones (*51*). A variety of genes have been isolated whose expression is cycle-dependent (*52*). The ability of a yeast cell to switch its mating type is transmitted specifically and predictably to one of two daughter cells and is a clear example of cell determination. This is accomplished in *Saccharomyces cerevisiae* by a gene whose expression is regulated by the cell cycle (*53*).

Although we are persuaded that cell cycle–dependent synthesis of regulatory factors, especially those involved in cell commitment, will be generally important for many genes, our recent experiments summarized here suggest that it is not necessary to involve cell cycle–dependent control of 5S DNA transcription factors to account for the differential control of oocyte and somatic 5S DNA in somatic cells. The replication of 5S RNA genes in somatic cells abides by the general rule (*54*) that active (somatic) 5S RNA genes are replicated early in S phase, while inactive (oocyte) 5S RNA genes are replicated late (*55*). It has been proposed that the early replicating somatic genes might deplete a limiting transcription factor such as TFIIIA (*56*). However, our data suggest that a high concentration of TFIIIA is required but insufficient to program oocyte 5S RNA genes in somatic cells (*49*). Elevation of limiting factors is predicted to program the oocyte 5S RNA genes transiently at S phase, but then, because chromatin matures after replication, the inherent instability of transcription complexes associated with oocyte 5S RNA genes would inevitably lead to their inactivation without the need for cell cycle–dependent control of transcription factor excess.

Developmental Regulation

These considerations concerning stable inactive and active states of genes are important as we reconstruct how they come to be imposed on the two 5S RNA genes in *X. laevis* during development.

Oogenesis. Growing *Xenopus* oocytes synthesize and accumulate ribosomes at rates that exceed that of the most active somatic cell by several orders of magnitude. The genetic mechanism for this enhanced rate of synthesis differs for each ribosome component. Thus, the genes for 18S and 28S ribosomal RNA are amplified only in oocytes (*57*), whereas the genes for 5S RNA have a large auxiliary multigene family that is transcribed only in oocytes and then is repressed in somatic cells where the demand for 5S RNA is reduced.

Synthesis of 5S RNA begins early in oogenesis and oocyte-type 5S RNA accumulates to high levels in oocytes months before ribosomes are assembled (*58*). The 5S RNA is stored in two ribonucleoprotein particles (*59*) during this early period, associated with two related 5S RNA binding proteins, one of which is TFIIIA (*60*). Evolution has fashioned this second use for TFIIIA, namely, the storage of 5S RNA before ribosome assembly. Free TFIIIB and TFIIIC are also present since extracts of oocyte nuclei transcribe exogenous transfer RNA and 5S RNA genes with high efficiency

and assemble stable complexes on exogenous oocyte and somatic 5S RNA genes. Transcription factor excess accounts partially for the very high rate of 5S RNA synthesis and the activation of the large oocyte-specific 5S RNA multigene family during oogenesis.

Oocyte and somatic 5S RNA genes are transcribed with comparable efficiency under conditions of transcription factor excess. The relative transcription efficiency (S/O ratio) of cloned oocyte and somatic 5S RNA genes that are injected into oocyte nuclei is about four (*46, 61*), a value similar to that observed when the two genes are transcribed in extracts from oocyte nuclei (*62*). By increasing DNA concentration or lowering TFIIIA concentration in extracts of oocyte nuclei, the S/O ratio has never been made to exceed ten. If the gene concentration is reduced to very low levels then both oocyte and somatic 5S DNA can be transcribed very efficiently (*27*) (300 transcripts per gene per hour), the rate that is estimated to occur in vivo (*63*).

Meiosis. When an oocyte matures either in vivo (*64*) or as a result of progesterone administration to oocytes in culture (*65, 66*), nuclear RNA transcription from endogeneous genes is repressed. Likewise, cloned somatic and oocyte 5S RNA genes injected into oocyte nuclei are inactivated after breakdown of the oocyte nucleus at meiosis (*42*). As predicted from this finding, genes that are added at a low concentration and that are fully active when either injected into oocyte nuclei or transcribed in extracts of oocyte nuclei, are inactive when injected into unfertilized eggs or transcribed in extracts of these eggs in vitro (*23, 46*). Transcription factors are actually abundant in eggs after meiosis, but their effective concentration is greatly reduced by virtue of unidentified inhibitors in eggs. High concentrations of nonspecific DNA overcome this inhibition. Oocyte 5S RNA genes are transcribed especially inefficiently in eggs (*46*) and egg extracts (*23*) compared to somatic 5S RNA genes (S/O ratio of 50).

Studies of transcription in the egg extract in vitro demonstrate two reasons for the inactivity of 5S RNA genes in eggs. First, RNA polymerase III that was located in the oocyte nucleus becomes mixed with the egg cytoplasm at meiosis. The polymerase is still active but clearly inhibited by the egg cytoplasm. Second, oocyte but not somatic 5S RNA gene transcription complexes are destabilized in egg extracts in contrast to their stability in oocyte nuclear extracts (*23*). Since stability of a complex is conferred by TFIIIA and TFIIIC, the destabilization is presumed to influence the joint interaction of these two factors with the ICR. This in turn must affect TFIIIB binding, which does not occur in the absence of the first two factors (*21, 25*).

Experiments with an extract of whole oocytes [oocyte S150 (*67*)] in which TFIIIA is in excess (*68*), revealed that oocyte and somatic 5S RNA genes can also be differentially transcribed (*69*). TFIIIC is limiting in this extract (*70*), so oocyte and somatic 5S RNA genes are differentially transcribed even when TFIIIA is bound to the ICR (*69*). Thus, transcription from oocyte 5S RNA genes becomes dependent on the concentration of any one of the factors in the extract. Because of complex instability, transcription of the oocyte 5S RNA genes is more sensitive to factor concentration than is the transcription of somatic 5S RNA genes. The S/O transcription ratio can range from unity to 500 simply by raising or lowering the TFIIIA concentration in the egg extract. The endogenous TFIIIA concentration in egg extract supports an intermediate S/O transcription ratio of 50. This is the same transcription ratio of the endogenous 5S RNA genes when they are first activated at the 4000-cell stage [midblastula transition (MBT)] of embryogenesis (*66, 71*).

Cleavage. After fertilization, the *Xenopus* egg undergoes 12 cycles of cell division (cleavage) very rapidly without G_1 or G_2 periods of the cell cycle (*72*). This rapid rate of cleavage certainly plays a role in the apparent transcriptional inactivity of all genes including 5S RNA genes (*73*). Mixtures of oocyte and somatic 5S RNA genes injected into cleaving embryos are transcribed with an S/O ratio of 50, which shows that the factors for 5S RNA gene transcription are present even when endogenous genes are not being transcribed. Each cycle of replication is expected to displace transcription factors from genes practically as soon as they are bound. This would prevent the efficient assembly of functional 5S RNA gene transcription complexes, because the lag period for functional transcription complex assembly on 5S RNA genes injected into cleaving embryos appears to be over 45 min (*46*). In contrast to endogenous DNA, if a large amount of DNA is injected into rapidly cleaving embryos, it is not efficiently replicated, so that there is time to form transcription complexes. Transcriptional activation of endogenous genes at the midblastula stage (the MBT) is concomitant with lengthening of the cell cycle and the appearance of G_1 and G_2, which gives time for transcription complexes to form. If DNA replication is inhibited in vivo before the MBT, transcriptional activation occurs (*73*). Thus, simple logistical features may play significant roles in the generalized inhibition of transcription that occurs during the period of rapid cleavage.

Gastrulation. At the MBT, oocyte 5S RNA transcription is low as a consequence of both the TFIIIA concentration and the instability of oocyte 5S RNA gene transcription complexes. A substantial fraction of the oocyte 5S RNA genes are already repressed by chromatin containing histone H1. By raising the amount of TFIIIA before MBT, transcription of oocyte 5S RNA genes (and to a lesser extent somatic 5S RNA genes) can be greatly elevated at MBT and through early gastrulation (*46, 49*). This activation of oocyte 5S RNA gene transcription can occur in the absence of DNA replication.

The period of responsiveness to high levels of TFIIIA is transient, ending at late gastrulation. After this time, the oocyte 5S RNA genes are inactivated progressively until the S/O ratio of both normal and TFIIIA enhanced embryos is about 1000 by early neurulation. The cessation of oocyte 5S RNA gene transcription (*49*), just like its stimulation (*46*), can occur in the absence of DNA replication. This loss of activity is attributable to the selective loss of transcription complexes associated with oocyte 5S RNA genes caused by the instability of these complexes. When complex instability occurs, repression of the oocyte 5S RNA genes is inevitable. Genes without transcription complexes become stably repressed by the assembly of chromatin containing histone H1. The stability of this repression dictates the concentration of factors needed to reactivate the gene. Since transcription factors remain associated with somatic 5S RNA genes, these genes resist the formation of repressed chromatin structures throughout embryogenesis. Conceivably the intrinsic stability of repression changes between MBT and neurulation. As the cell cycle lengthens, regions of interphase chromatin may become more compacted and much less accessible to exogenous transcription factors or less available for histone H1 exchange. This may explain why high levels of TFIIIA activate oocyte 5S RNA genes at MBT but not after gastrulation.

The process outlined above details the establishment of a pattern of gene activity whose end result is transcription complexes assembled on the somatic 5S RNA genes and a stable repressed chromatin structure on the closely related oocyte 5S RNA genes in the same cell (Fig. 4).

In earlier models (*28, 46*) we postulated that different binding affinities of TFIIIA to the two kinds of 5S RNA genes and changes in concentration of TFIIIA during development could account for part but not all of the exaggerated differential gene action that ultimately occurs in somatic cells. In light of recent experiments summarized in this article, we now believe that the essential property that is required for this differential gene expression is the

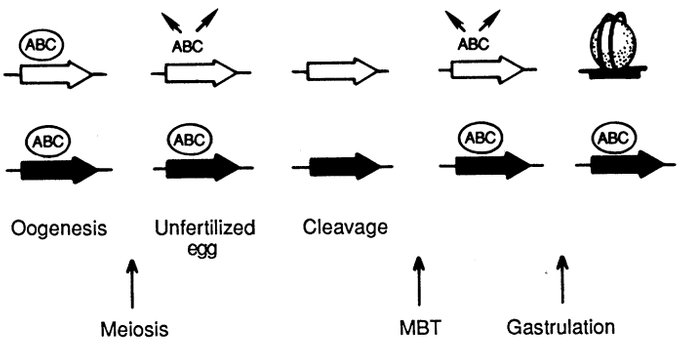

Fig. 4. Model for developmental control of 5*S* RNA gene transcription. This diagram summarizes the occupancy of oocyte (upper) and somatic (lower) 5*S* DNA with transcription complexes or chromatin during oogenesis and embryogenesis. A stable complex is represented by the factors A, B, and C encircled. The loss of the circle around a complex along with the arrows indicates unstable transcription complexes. These genes are still accessible to high levels of factors. The end result by late gastrulation is stable transcription complexes assembled on somatic 5*S* RNA genes and a repressed chromatin structure on oocyte 5*S* DNA.

differential stability of the two kinds of transcription complexes in somatic cells. Three of the six base differences between oocyte and somatic 5*S* RNA genes contribute to differential transcription complex stability in vitro (*23*); they are located within the 5′ part of the ICR. Their effect on the binary reaction between TFIIIA and the ICR is minimal (*74*), so we presume that it is the stabilization of the binding of TFIIIA by TFIIIC that is involved in this differential stability. This will not be definitively established until all of the transcription factors involved in 5*S* RNA gene activity are purified and characterized. It must be pointed out also that experiments in vivo have not been reported where cloned somatic and oocyte 5*S* RNA genes are brought under the same differential gene expression as the endogenous genes. Such an assay is still needed to confirm the indication that the exaggerated S/O of 1000 characteristic of somatic cells is attributable to just 3 base pairs within the ICR.

Conclusion

These studies lead to a simple model for the developmental regulation of two closely related genes. The model relies on progressive limitation of transcription factors during development coupled with a difference in the stability of transcription complexes that these factors form with the promoters (internal control regions) of the two 5*S* RNA genes. When oocyte 5*S* RNA genes become unoccupied by transcription factors a repressed chromatin structure forms that can then exclude the subsequent binding of the factors. This repressive structure depends on the interaction of histone H1 with nucleosomes. The repression of genes by nucleosomes and histone H1 appears to be opportunistic. Whatever DNA region is not assembled into active transcription complexes will be compacted into repressed chromatin. When a DNA region is programmed into an active transcription complex, this serves to interrupt the repetitive positioning of nucleosomes that is needed for chromatin to compact into higher order repressive structures. We propose that a decision to activate or repress a gene must be made each time the gene is replicated because DNA replication removes even stably complexed proteins from control regions of a gene, making them accessible to either activating factors or compaction into repressed chromatin.

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78. We thank E. Jordan for technical assistance and E. Crawford, M. Darby, J. Gall, S. Kim, D. Koshland, S. McKnight, A. Spradling, S. Ward, and Y. Yaoita for comments. A.P.W. was supported by a long-term fellowship from the European Molecular Biology Organization and a grant from the American Cancer Society (Maryland division). Supported in part by NIH grant GM22395.

Research Articles

The SCID-hu Mouse: Murine Model for the Analysis of Human Hematolymphoid Differentiation and Function

J. M. McCUNE, R. NAMIKAWA, H. KANESHIMA, L. D. SHULTZ, M. LIEBERMAN, I. L. WEISSMAN

The study of human hematopoietic cells and the human immune system is hampered by the lack of a suitable experimental model. Experimental data are presented showing that human fetal liver hematopoietic cells, human fetal thymus, and human fetal lymph node support the differentiation of mature human T cells and B cells after engraftment into mice with genetically determined severe combined immunodeficiency. The resultant SCID-hu mice are found to have a transient wave of human CD4$^+$ and CD8$^+$ T cells and human IgG (immunoglobulin G) in the peripheral circulation. The functional status of the human immune system within this mouse model is not yet known.

TO APPROXIMATE THE EVALUATION OF DISEASE STATES IN man, biomedical research has relied heavily upon animal models. Of these, experiments with the laboratory mouse have contributed much to our understanding of the immune system, the cells involved, the products that they express, and their differentiation pathways. After immunization in vivo, murine splenic B cells can be immortalized as hybridoma lines making monoclonal antibodies (*1*), and functional, antigen-reactive murine T cells can easily be cloned (*2*). After adoptive transfer into lethally irradiated hosts, the murine pluripotent hematopoietic stem cell can be identified, purified, and studied (*3, 4*). These are important findings. They are not, however, easily or directly applicable to man; except in rare circumstances, humans cannot be subjected to experimental immunizations or to lethal irradiation and could not provide internal lymphoid organs.

The emergence of the acquired immunodeficiency syndrome (AIDS) underscores the need for direct and comprehensive analysis of the human immune system (*5*). With this epidemic, as well as with those associated with other human lymphotropic retroviruses, little is known about the course of infection in vivo. In the absence of a testable model, the accumulation of knowledge may be slow. Clinical trials represent the only available means of evaluating therapeutic or prophylactic modalities. Although similar animal retroviruses are associated with immunodeficiency states, in no case is the virus identical to human immunodeficiency virus or the disease identical to AIDS. Indeed, if any pertinent knowledge has been gained from the study of animal retroviruses, it is that retroviruses affecting man are best (and perhaps only) studied in the context of human, and not animal cells.

We have taken an alternative approach, one that might create an animal model precisely for the study of the human immune system, its physiology, and its pathophysiology. We now present a method by which the human T and B cell lineages and their hematopoietic precursors can be obtained, transplanted, and observed to differentiate within a mouse.

Several key concepts of immunology guide these experiments.

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