Année de publication : 2001

P Ridgway, G Almouzni (2001 Oct 31)
Chromatin assembly and organization.
Journal of cell science : 2711-2

Résumé

B Palancade, S Bellier, G Almouzni, O Bensaude (2001 Sep 18)
Incomplete RNA polymerase II phosphorylation in Xenopus laevis early embryos.
Journal of cell science : 2483-9

Résumé

Phosphorylation of RNA polymerase II largest subunit on its C-terminal domain (CTD) heptapeptide repeats has been shown to play a key role in the regulation of mRNA synthesis and processing. In many higher metazoans, early embryos do not synthesise mRNAs during the first cell cycles following fertilisation. Transcription resumes and becomes an absolute requirement for development after several cell cycles characteristic of each species. Therefore, CTD phosphorylation has been investigated during early development of the African clawed-frog Xenopus laevis. Fertilisation is shown to trigger an abrupt dephosphorylation of the CTD. Phosphorylation of the CTD resumes concurrently with the mid-blastula transition (MBT). Both are advanced with polyspermy and increased temperatures; they do not occur when replication is impaired with aphidicolin. In Xenopus laevis somatic cells, a set of monoclonal antibodies defined distinct phosphoepitopes on the CTD. Two of them were absent before the MBT indicating that the CTD lacks the phosphorylation at the serine-2 position of the heptapeptide. The possible contribution of RNA polymerase II phosphorylation to the developmental-regulation of maternal mRNA processing in embryos is discussed.

G Almouzni (2001 Sep 1)
The post-doc's view.
Journal of cell science : 3074

Résumé

C Verheggen, S Le Panse, G Almouzni, D Hernandez-Verdun (2001 Aug 30)
Maintenance of nucleolar machineries and pre-rRNAs in remnant nucleolus of erythrocyte nuclei and remodeling in Xenopus egg extracts.
Experimental cell research : 23-34

Résumé

The nuclear functions in erythrocytes are almost completely extinct. There is no RNA polymerase I transcription, although a remnant nucleolar structure is still present. The remnant nucleolus of Xenopus laevis erythrocytes maintains a morphologically organized structure, nearly exclusively fibrillar. In this inactive nucleolar remnant, we revealed the presence of a modified form of transcription factor UBF. Several proteins of the processing machinery such as fibrillarin, nucleolin and B23/NO38, snoRNAs U3 and U8, and partially processed preribosomal RNAs colocalized in these remnant structures. Attempts to reprogram these erythrocyte nuclei in Xenopus egg extract showed that import of several nucleolar proteins was induced while the nucleolar remnant was disorganized. UBF became abundant and showed a necklace-like distribution on the decondensed ribosomal genes. Fibrillarin, nucleolin, and snoRNAs U3 and U8, also largely imported from the extract, were associated in large prenuclear bodies scattered in the nucleoplasm. B23/NO38 was present in different small bodies formed only in the most decondensed nuclei. In these remodeled erythrocyte nuclei, there was no imported preribosomal RNA and the initial presence of a residual nucleolar structure containing several partners of ribosome biogenesis was not sufficient to promote reassembly of newly imported nucleolar machineries. These nuclei, which reproduce the early events of nucleogenesis are also transcriptionally silent and thus compare to the early embryonic nuclei of Xenopus laevis.

M Brand, J G Moggs, M Oulad-Abdelghani, F Lejeune, F J Dilworth, J Stevenin, G Almouzni, L Tora (2001 Jun 19)

UV-damaged DNA-binding protein in the TFTC complex links DNA damage recognition to nucleosome acetylation.

The EMBO journal : 3187-96

Résumé

Initiation of transcription of protein-encoding genes by RNA polymerase II (Pol II) was thought to require transcription factor TFIID, a complex comprised of the TATA box-binding protein (TBP) and TBP-associated factors (TAF(II)s). In the presence of TBP-free TAF(II) complex (TFTC), initiation of Pol II transcription can occur in the absence of TFIID. TFTC containing the GCN5 acetyltransferase acetylates histone H3 in a nucleosomal context. We have identified a 130 kDa subunit of TFTC (SAP130) that shares homology with the large subunit of UV-damaged DNA-binding factor. TFTC preferentially binds UV-irradiated DNA, UV-damaged DNA inhibits TFTC-mediated Pol II transcription and TFTC is recruited in parallel with the nucleotide excision repair protein XP-A to UV-damaged DNA. TFTC preferentially acetylates histone H3 in nucleosomes assembled on UV-damaged DNA. In agreement with this, strong histone H3 acetylation occurs in intact cells after UV irradiation. These results suggest that the access of DNA repair machinery to lesions within chromatin may be facilitated by TFTC via covalent modification of chromatin. Thus, our experiments reveal a molecular link between DNA damage recognition and chromatin modification.

J P Quivy, P Grandi, G Almouzni (2001 Apr 11)
Dimerization of the largest subunit of chromatin assembly factor 1: importance in vitro and during Xenopus early development.
The EMBO journal : 2015-27

Résumé

To date, the in vivo importance of chromatin assembly factors during development in vertebrates is unknown. Chromatin assembly factor 1 (CAF-1) represents the best biochemically characterized factor promoting chromatin assembly during DNA replication or repair in human cell-free systems. Here, we identify a Xenopus homologue of the largest subunit of CAF-1 (p150). Novel dimerization properties are found conserved in both Xenopus and human p150. A region of 36 amino acids required for p150 dimerization was identified. Deletion of this domain abolishes the ability of p150 to promote chromatin assembly in vitro. A dominant-negative interference based on these dimerization properties occurs both in vitro and in vivo. In the embryo, nuclear organization was severely affected and cell cycle progression was impaired during the rapid early cleaving stages of Xenopus development. We propose that the rapid proliferation at early developmental stages necessitates the unique properties of an assembly factor that can ensure a tight coupling between DNA replication or repair and chromatin assembly.

J A Mello, G Almouzni (2001 Mar 16)
The ins and outs of nucleosome assembly.
Current opinion in genetics & development : 136-41

Résumé

De novo nucleosome assembly coupled to DNA replication and repair in vitro involves the histone chaperone chromatin assembly factor 1 (CAF-1). Recent studies support a model in which CAF-1 can be targeted to newly synthesized DNA through a direct interaction with proliferating cell nuclear antigen (PCNA) and can act synergistically with a newly identified histone chaperone. Insights have also been obtained into mechanisms by which this CAF-1-dependent pathway can establish a repressed chromatin state.

A Taddei, C Maison, D Roche, G Almouzni (2001 Feb 15)
Reversible disruption of pericentric heterochromatin and centromere function by inhibiting deacetylases.
Nature cell biology : 114-20

Résumé

Histone modifications might act to mark and maintain functional chromatin domains during both interphase and mitosis. Here we show that pericentric heterochromatin in mammalian cells is specifically responsive to prolonged treatment with deacetylase inhibitors. These

defined regions relocate at the nuclear periphery and lose their properties of retaining HP1 (heterochromatin protein 1) proteins. Subsequent defects in chromosome segregation arise in mitosis. All these changes can reverse rapidly after drug removal. Our data point to a crucial role of histone underacetylation within pericentric heterochromatin regions for their association with HP1 proteins, their nuclear compartmentalization and their contribution to centromere function.

Année de publication : 2000

B Ladoux, J P Quivy, P Doyle, O du Roure, G Almouzni, J L Viovy (2000 Dec 13)

Fast kinetics of chromatin assembly revealed by single-molecule videomicroscopy and scanning force microscopy.

Proceedings of the National Academy of Sciences of the United States of America : 14251-6

Résumé

Fluorescence videomicroscopy and scanning force microscopy were used to follow, in real time, chromatin assembly on individual DNA molecules immersed in cell-free systems competent for physiological chromatin assembly. Within a few seconds, molecules are already compacted into a form exhibiting strong similarities to native chromatin fibers. In these extracts, the compaction rate is more than 100 times faster than expected from standard biochemical assays. Our data provide definite information on the forces involved (a few piconewtons) and on the reaction path. DNA compaction as a function of time revealed unique features of the assembly reaction in these extracts. They imply a sequential process with at least three steps, involving DNA wrapping as the final event. An absolute and quantitative measure of the kinetic parameters of the early steps in chromatin assembly under physiological conditions could thus be obtained.

P Ridgway, G Almouzni (2000 Jul 13)

CAF-1 and the inheritance of chromatin states: at the crossroads of DNA replication and repair.

Journal of cell science : 2647-58

Résumé

Chromatin is no longer considered to be a static structural framework for packaging DNA within the nucleus but is instead believed to be an interactive component of DNA metabolism. The ordered assembly of chromatin produces a nucleoprotein template capable of epigenetically regulating the expression and maintenance of the genome. Factors have been isolated from cell extracts that stimulate early steps in chromatin assembly in vitro. The function of one such factor, chromatin-assembly factor 1 (CAF-1), might extend beyond simply facilitating the progression through an individual assembly reaction to its active participation in a marking system. This marking system could be exploited at the crossroads of DNA replication and repair to monitor genome integrity and to define particular epigenetic

states.

P H Gaillard, D Roche, G Almouzni (2000 May 11)
Nucleotide excision repair coupled to chromatin assembly.
Methods in molecular biology (Clifton, N.J.) : 231-43

Résumé

P Ridgway, J P Quivy, G Almouzni (2000 Apr 25)
Tetracycline-regulated gene expression switch in Xenopus laevis.
Experimental cell research : 392-9

Résumé

Xenopus is a well-characterized model system for the investigation of biological processes at the molecular, cellular, and developmental level. The successful application of a rapid and reliable method for transgenic approaches in Xenopus has led to renewed interest in this system. We have explored the applicability of tetracycline-regulated gene expression, first described by Gossen and Bujard in 1992, to the Xenopus system. By optimizing conditions, tetracycline repressor induced expression of a luciferase reporter gene was readily and reproducibly achieved in both the Xenopus oocyte and developing embryo. This high level of expression was effectively abrogated by addition of low levels of tetracycline. The significance of this newly defined system for studies of chromatin dynamics and developmental processes is discussed.

C Verheggen, G Almouzni, D Hernandez-Verdun (2000 Apr 18)
The ribosomal RNA processing machinery is recruited to the nucleolar domain before RNA polymerase I during Xenopus laevis development.
The Journal of cell biology : 293-306

Résumé

Transcription and splicing of messenger RNAs are temporally and spatially coordinated through the recruitment by RNA polymerase II of processing factors. We questioned whether RNA polymerase I plays a role in the recruitment of the ribosomal RNA (rRNA) processing machinery. During Xenopus laevis embryogenesis, recruitment of the rRNA processing machinery to the nucleolar domain occurs in two steps: two types of precursor structures called prenucleolar bodies (PNBs) form independently throughout the nucleoplasm; and components of PNBs I (fibrillarin, nucleolin, and the U3 and U8 small nucleolar RNAs) fuse to the nucleolar domain before components of PNBs II (B23/NO38). This fusion process is independent of RNA polymerase I activity, as shown by actinomycin D treatment of embryos and by the lack of detectable RNA polymerase I at ribosomal gene loci during fusion. Instead, this process is concomitant with the targeting of maternally derived pre-rRNAs to the

nucleolar domain. Absence of fusion was correlated with absence of these pre-rRNAs in nuclei where RNA polymerase II and III are inhibited. Therefore, during X. laevis embryogenesis, the recruitment of the rRNA processing machinery to the nucleolar domain could be dependent on the presence of pre-rRNAs, but is independent of either zygotic RNA polymerase I transcription or the presence of RNA polymerase I itself.

S Belikov, B Gelius, G Almouzni, O Wrange (2000 Mar 4)
Hormone activation induces nucleosome positioning in vivo.
The EMBO journal : 1023-33

Résumé

The mouse mammary tumor virus (MMTV) promoter is induced by glucocorticoid hormone. A robust hormone- and receptor-dependent activation could be reproduced in Xenopus laevis oocytes. The homogeneous response in this system allowed a detailed analysis of the transition in chromatin structure following hormone activation. This revealed two novel findings: hormone activation led to the establishment of specific translational positioning of nucleosomes despite the lack of significant positioning in the inactive state; and, in the active promoter, a subnucleosomal particle encompassing the glucocorticoid receptor (GR)-binding region was detected. The presence of only a single GR-binding site was sufficient for the structural transition to occur. Both basal promoter elements and ongoing transcription were dispensable. These data reveal a stepwise process in the transcriptional activation by glucocorticoid hormone.

J G Moggs, P Grandi, J P Quivy, Z O Jónsson, U Hübscher, P B Becker, G Almouzni (2000 Jan 29)
A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage.
Molecular and cellular biology : 1206-18

Résumé

Sensing DNA damage is crucial for the maintenance of genomic integrity and cell cycle progression. The participation of chromatin in these events is becoming of increasing interest. We show that the presence of single-strand breaks and gaps, formed either directly or during DNA damage processing, can trigger the propagation of nucleosomal arrays. This nucleosome assembly pathway involves the histone chaperone chromatin assembly factor 1 (CAF-1). The largest subunit (p150) of this factor interacts directly with proliferating cell nuclear antigen (PCNA), and critical regions for this interaction on both proteins have been mapped. To isolate proteins specifically recruited during DNA repair, damaged DNA linked to magnetic beads was used. The binding of both PCNA and CAF-1 to this damaged DNA was dependent on the number of DNA lesions and required ATP. Chromatin assembly linked to the repair of single-strand breaks was disrupted by depletion of PCNA from a cell-free system. This defect was rescued by complementation with recombinant PCNA, arguing for role of PCNA in mediating chromatin assembly linked to DNA repair. We discuss the importance of the PCNA-CAF-1 interaction in the context of DNA damage processing and

checkpoint control.