The Mechanisms of Pyknosis: Hypercondensation and Death

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Intense nuclear condensation with intense refractivity (pyknosis) is the ubiquitous terminus of all apoptosis and some necrosis of vertebrate cells, but its structural basis is unknown. Intense condensations were induced in a model system, the avian erythrocyte, and three different molecular processes distinguished from each other. Two of the hypercondensations, nucleolytic pyknosis, as in mammalian apoptosis, and anucleolytic pyknosis, as in necrosis, appear to be energetically spontaneous and appear to have a conformational basis with the third hypercondensation being a trans-nuclear membrane osmotic pressure compression effect. Nucleolytic pyknosis as per apoptosis was not intrinsic in this system and required exogenous nuclease. The pure anucleolytic pyknosis supported by this system was not induced by the apoptopic induction agents, staurosporine or antitopoisomerases (I and II), indicating a simple but unusual signaling pathway for anucleolytic pyknosis. Molecular weight determinations of the H5, H3, H4, H2a, and H2b, with final errors of ±1 Da or less, seem to eliminate histone modifications as the basis of nucleolytic pyknosis. The molecular basis of pyknosis is proposed to be from internucleosomal rotational angle freedom that permits internucleosomal sharing of basic histone tails of adjacent nucleosomes and nucleofilaments. Much of the favorable conformational energy of pyknosis may be from the entropy increase of tail delocalization.

Key Words: chromatin; pyknosis; apoptosis; necrosis; histones; avian erythrocyte.

INTRODUCTION

Eukaryotic chromatin has many condensation states characteristic of its various activities. These range from the decondensed chromatin of highly active genes, to moderately condensed interphase chromatin, to much more condensed mitotic chromatin, down to the intensely condensed pyknotic state found in dying and dead cells. Most living nuclei are mixtures of many of these states but the avian erythrocyte provides a model system with what appears to be two relatively pure states of condensation and a brief transition between the two. The first, the normal state of these cells, may be described as a relatively normal but condensed transcriptionally inactive interphase chromatin but the second state, the pyknotic state, is so intensely condensed that it is at the physical limit of condensation. A true limit exists when condensing chromatin has no usable space left within it because the nucleosomes are close packed and further condensation is, obviously, physically impossible. Electron microscope sections of this chromatin shows that this is indeed the case with erythrocyte pyknosis (see figures in this article). The avian erythrocyte nucleus comes up against this natural limit of pyknosis when the hypercondensing chromatin has approximately one-third of the volume of the normal, transcriptionally inactive interphase chromatin.

Pyknosis is not peculiar to avian erythrocytes as it is an extremely familiar state of condensation found in almost all dying cells and is the most characteristic of all expression of programmed cell death. Pyknosis is interesting for two reasons. It is structurally informative in that its understanding seems highly relevant to the recalcitrant problems of nucleosomal packing in translationally inactive chromatin. However, pyknosis is equally important in understanding cell death because all higher organisms seem to be capable of it and, yet, no living states have been reported as showing it. With most vertebrate cells the most distinctive aspect of death is this physical collapse of the nucleus called pyknosis or "hypercondensation of chromatin" so much so that pyknosis is the accepted symptom of programmed death [e.g., 1–3].

Cooperation of the opposing processes of cell death and division forms and maintains tissues and appropriate cell deletion is a major bulwark against cancer progression while inappropriate cell deletion is the basis of some disease states. Cell deletion by programmed death is not a single process. The term apoptosis tends to be reserved for the most complex of the programmed deaths, requiring ATP energy and transcription, but much simpler programs exist. In the simpler cases, pyknosis still occurs and much of the death program must be implicit in the structure of the macromolecules involved, for example, in the proforms of proteases or nucleases and/or implicit within the structure of chro-
mation by mass spectroscopy with a level of accuracy sufficient to detect any variations in histones such as demodifications or slight proteolysis. These results together with others were then used to infer molecular mechanism.

METHODS

PBS–EDTA is 153.09 mM Na<sup>+</sup>, 5.1687 mm K<sup>+</sup>, 10.6 mM phosphate, 139.6 chloride, 0.3 mM Tris, 0.1 mM EDTA, pH 7.5 HAE–Nonidet buffer, a derivative of the previously characterized nuclear structure maintenance salts, is 60 mM KCl, 15 mM NaOH, 0.15 mM Hepes (free acid), 0.15 mM spermine, 0.5 mM spermidine–HCl, 0.1 mM Na<sup>+</sup>–EDTA, 0.05% Nonidet P-40, 15 mM NaOH, adjusted to pH 7.5 with HCl (usage refs cited in [8]). Pyknotic and normal cells were lysed with HAE–Nonidet to retain original refractive differential and morphological differences.

Starvation induction: Protocol. EDTA-anticoagulated blood from Gallus gallus, inbred Ancona strain, was added to 15 vol of PBS–EDTA, washed twice more, suspended at an appropriate hematocrit, and observed over 0–52 h. Control (fed) cells were washed in the same way but suspended in PBS–EDTA, 0.2% glucose. Culture of washed cells with or without glucose, never exceeded 52 h because after 52 h cytoplasmic damage began developing in all cells. Glucose starvation was performed at 37°C, in air (it is very important that there is no supplementary CO<sub>2</sub>).

Acute ionophore toxicity conditions. Toxicity was induced with a 2-h incubation at 37°C in air in PBS–EDTA with 4 μg/ml gramicidin. Cell pellets were lysed in air in HAE–Nonidet buffer and after centrifugation, the lysate containing oxyhemoglobin and methemoglobin had both forms calculated from the optic density taken at four wavelengths: 500, 541.5, 561, and 577 nm.

Transmission EM. Fixation and embedding procedures were from [9].

Visible microscopy. Figures cited as “high power” were from a Nikon Diaphot 300 inverted with Nomarski contrast. Figures cited as “low power” were from a standard Olympus (obj = 40X, filter = PH2).

RESULTS

Data collection. Degrees of pyknosis clearly exist in the early stages of pyknosis but for the sake of objectivity, it was routinely scored (+) or (−) when the phase contrast microscopy showed any degree of pyknosis. Appropriate qualification such as vacuolation or the alternative, invagination, was noted from light microscopy of nuclei from lysed cells and the reality of this level of detail was confirmed from EM sections. When scored, nuclear morphology was readily classified as (a) nonpyknotic (normal), (b) marginalized pyknotic, or (c) nonmarginalized, concave invaginated pyknotic. Initially it was still uncertain whether marginalized pyknotic morphology was nucleolytic or nonnucleolytic so DNA was extracted and examined. However, nucleolytic pyknosis was never observed in these cells except when exogenous nucleases were added. Similarly, concave invaginations were not observed unless ionophore toxicity was involved.

Statistical analysis was not required for scoring as the marked uniformity of response of these uniform, noncycling cells made it unnecessary. Changes of state...
were usually scored for a whole microscope field as change usually involved most cells in a sample except briefly during transitions from one state to another and these transitional periods were not relevant to the issues this article addresses. Trypan blue penetration of the cells was observed to occur at the same time as total hemoglobin release; thus total lysis was objectively quantified from the heme spectra of cell lysates. Intact cells were represented by the total hemoglobin (deoxy + oxy hemoglobin + methemoglobin) retained in a cell pellet.

Normal cells and nuclei. In PBS–EDTA the normal cells were pink, ellipsoid cells with a central, diffuse nucleus of similar refractive index to the cytoplasm (e.g., Fig. 1A). A very few spontaneously pyknotic cells were always observable in the normal population but in fresh blood from healthy birds the numbers were always negligible (less than 1%). The normal cells mostly showed very small amounts of nuclear vacuolation (see an electron microscope section of a cell, Fig. 2A, and a electron micrograph of an isolated, normal, nucleus, Fig. 4A).

In visible light the nuclei in the normal, unstarved cells are invisible although a large, nucleus-like oval zone over them gives an illusion of a nucleus as shown in Fig. 1A. This zone, however, corresponds to a cytoplasmic outbulge surrounding the nucleus, not the nucleus inside it. Because the large, nonpyknotic nuclei had a similar refractive index to their cytoplasm, the unstained normal nucleus was only observable in visible light in HAE–Nonidet lysates and then were indistinct. Figure 3A shows a low power, phase contrast field of nuclei rescued just before pyknotic transition. Unstarved cells held in their original blood at 4°C do not become strongly pyknotic until approximately 4 weeks.

Anucleolytic pyknosis. DNA from pyknotic nuclei from starved cells was examined for DNA breaks on neutral agarose gel, pulsed field gel, and alkaline agarose gels. No differences between pyknotic and control DNA were seen. The only DNA breaks observed from populations of starvation-pyknotosed nuclei were trivial and had variable amount of fragments, of the size and frequency expected from the very minor and variable presence of apoptotic white cells contaminating erythrocyte preparations. The overwhelming bulk of the DNA had a size in accord with the handling procedures and resolution of procedures used to examine it. Very conservatively this means that whatever breaks were present were only single stranded and then more than 20 kb apart. Single-stranded breaks of this low order of frequency are explainable as dynamic topoisomerase intermediate states and the break levels reported in true nucleolytic apoptosis are one or two orders of magnitude greater than this.

In blood stored at 4°C, pyknosis and lysis may take as long as 1 month but after PBS–EDTA washing fresh cells, pyknosis becomes irreversible after 14 to 20 h of glucose starvation. Figures 1A and 1B show a high power, visible light field of these nuclei just before and just after the pyknotic transition. Electron microscope sections in Figs. 2a and 2b show the cells before (Fig. 2A) and after (Fig. 2B) the pyknotic transition. It is noteworthy, as in Fig. 2b, that late pyknotic cells also begin to lose the standard ellipsoid shape of the normal cells’ cytoplasm. On lysis the pyknotic nuclei (Fig. 3B) are extremely refractive compared to immediately pyknotic nuclei.

Moreover, once clearly observable from glucose starvation, pyknosis is not rescued by glucose addition even though the pyknotic cells still exclude trypan blue and retain hemoglobin (Table 1). The question of whether nuclei caught anywhere at all in the very first stages of pyknosis may be rescued is an interesting question but there is negligible reversal by the time it is clearly observable.

Structural changes in living cells were monitored with phase optics, as the pyknotic nuclei of the starved cells increased refractivity and shrunk to a terminal size of approximately one-third of the original volume depending on the degree of vacuolation. Cell lysis in HAE–Nonidet did not change nuclear morphology and confirmed that much, or all, of the refractive differential was a nuclear property with little contribution from any changes in cytoplasmic refractiveness. The nuclear morphology, pyknotic or nonpyknotic, was stable in HAE–Nonidet lysates for at least 24 h at 4°C. The histone primary structure and modification state during anucleolytic pyknosis were determined by electrospray mass spectroscopy of the histones from normal and pyknotic nuclei. The technology was quite capable of detecting less than a difference of a single amino acid, a single phosphate, or a single acetate group per molecule. There was no indication of any such differences, and there were no changes in histone modifications nor partial proteolytic products. The sizes of the major histones agreed well with expectations from the known sequences of the primary transcripts and the only modifications indicated were acetylations and methylations (see Table 2). There was no indication of a basal level of phosphorylation of the major histones in this particular developmental state.

The only major protein difference detected in pyknotic nuclei was with two unidentified nonhistone proteins at 15,300 and at 32,674 Da. They were lost during the pyknotic transition. However, evidence indicated that their loss was consequential, not causal, as they could be extracted from nonpyknotic nuclei with dilute salt without triggering pyknosis.

The acetylation and methylation status of the major histones was readily apparent from their mass and both were unchanged by pyknosis. The H5 (20,605) and the H2B1 (13,793) were as expected for unmodified
histones. The molecular weights of both chicken H2A4 (13,852) and H4 (11,308) were expected values from known gene sequences, after allowing for the N-terminal acetylations expected for both these peptides and the dimethylation expected for H4 at Lys-20 [10]. In contrast to H5, H2B, and H2A, which had one major member each, the H3 was a cluster of peaks, of interpeak spacing of 14 Da representing a methylation family extending from a lower limit of monomethyl H3 to an ill-defined upper limit of more than eight methyls. There was negligible unmethylated H3 and the highest peak, before and after pyknosis, was always tetramethyl-H3 at 15,313 Da.

In summary, two unidentified proteins were lost during pyknosis and the major histones did not show any changes.

Elimination of the “plastic distortion” explanation for anucleolytic pyknosis. By themselves, the refractivity and morphological differences between normal and starved pyknotic nuclei suggested two possible explanations for condensation. The trivial explanation was that the collapse was a structurally unimportant, passive plastic distortion in response to an externally driven compression. The more structurally significant explanation was that condensation state was a macroscopic expression of molecular events within chromatin; i.e., the pyknosis was conformational in origin. A simple test for these alternatives was performed. If the passive plastic distortion model was the case, then forcible decondensation of nuclei with expansive buffers would distort the nuclei into an expanded, decondensed state and erase the original condensation state.
so that, on returning the nuclei to standard conditions in HAE, the nuclei would no longer remember their preexpansion state; ie., they were passively plastic. Conversely, if the molecular structure explanation were the case, the forcible physical expansion would not erase the nuclei’s memory of their original state.

Normal nuclei from unstarved cells and pyknotic nuclei from starved cells were forcibly decondensed with polyamine-free HAE buffer for a short period of time and then allowed to relax in HAE and examined by phase contrast. Forced decondensation was for 5 min in HAE minus the polyamines, in ice, and briefly forced the nuclei to be less condensed than the least condensed normal nuclei seen naturally. The normal polyamine levels of HAE were then restored and caused recondensation. It was observed that recondensed nuclei reverted to whatever their preexpansion state had been: condensed if they had been condensed and decondensed if they had been decondensed. The normal and pyknotic condensation states were thus remembered states and unaffected by the nuclei being artificially cycled through an extreme degree of apparent decondensation. This result is in accord with the state of the nuclei being determined by molecular substructure, i.e., conformation, not by simple passive plastic distortion.

### Starvation and the corresponding cytoplasmic changes (Fig. 1B).

The earliest evidence of change was the beginning of nuclear condensation as shown in Fig. 1B (compare control Fig. 1A). Later, the exhaustion of cytoplasmic reducing equivalents became visible to the naked eye from the cell suspension changing color from that of oxyhemoglobin to other forms of hemoglobin, mainly methemoglobin. (Note that there is evidence, not presented here, that indicates that hemoglobin responses to starvation are probably more complex than a simple conversion to methemoglobin.)

Late glucose starvation also causes an overall cell shape changed from an ellipsoid (normal shape) to a more irregular isometric shape as per Fig. 2B. There was also a cytoplasmic swelling in late starvation, implying a drop of cytoplasmic refractivity, but as noted, phase contrast after lysis as in contrast after lysis as in Fig. 3B compared to control Fig. 3A showed that changes in cytoplasmic refractiveness were not responsible for the bulk of the refractive difference between nucleus and cytoplasm. Cell death terminated with simple cytoplasmic lysis. There was no nuclear fragmentation like that of canonical apoptosis.

### TABLE 1

<table>
<thead>
<tr>
<th>Time of addition of glucose rescue (h)</th>
<th>Anucleolytic pyknosis apparent at 52 h</th>
<th>% Intact cells at 48.5 and 52 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>—</td>
<td>100, 96</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
<td>100, 91</td>
</tr>
<tr>
<td>3.5</td>
<td>—</td>
<td>100, 93</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>100, 93</td>
</tr>
<tr>
<td>14</td>
<td>± (Fig. 3A)</td>
<td>93, 75</td>
</tr>
<tr>
<td>20.5</td>
<td>+</td>
<td>96, 78</td>
</tr>
<tr>
<td>25.5</td>
<td>+ (Fig. 3B)</td>
<td>88, 71</td>
</tr>
<tr>
<td>32</td>
<td>+</td>
<td>91, 75</td>
</tr>
<tr>
<td>No rescue</td>
<td>+</td>
<td>—, 71</td>
</tr>
</tbody>
</table>

Note: Glucose rescue from pyknosis induction is possible until approximately 14 h of starvation. After 14 h, when pyknosis is just becoming visually apparent (Fig. 3A), nuclei were not rescued by glucose even though never less than 70% of the pyknotic cells have their outer membrane still intact. Washed cells were suspended in PBS–EDTA in a multiwell plate and lysis was monitored subjectively with trypan blue and objectively quantified from total hemoglobin retention. Pyknotic conversion occurred en masse when it took place and was thus scored as either + or − by phase optics. Glucose was added to a final concentration of 0.2% at the times indicated, in an attempt to stop and/or reverse nuclear pyknosis and/or reduce terminal lysis. Before approximately 14 h it did both and after 14 h there was no effect on pyknosis and a negligible effect on lysis.
Nucleolytic pyknosis. Figure 4B shows an example of an avian erythrocyte nucleus made nucleolytically pyknotic in vitro with a comparable but normal nucleus in Fig. 4A. Note the extreme marginilization and vacuolation and high density of the chromatin in Fig. 4B compared to Fig. 4A. This class of pyknosis has been extensively described previously, e.g., [1], and, as is shown in Fig. 4B, is also induced in avian erythrocyte nuclei in vitro with exogenous nucleases. The nucleolyzed chromatin pyknosed and marginalized to give large vacuoles as is commonly reported during apoptosis in many cell types. However, there was no endoge-

![Image](https://via.placeholder.com/150)

**FIG. 4.** Nucleolytic pyknosed nucleus: Transmission EM of a cross-section of a nucleolysed nucleus from an avian erythrocyte. (A) Normal, low-density, nonpyknotic nucleus. Nuclei from lysed, unstarved cells; shown are the relatively evenly dispersed chromatin and the very small amount of vacuolar space characteristic of a normal nucleus. Bar, 200 nm. (B) Nucleolytically pyknosed nucleus. A normal, low-density, nonpyknotic nucleus was exposed to DNase-I before fixation. Note the pronounced induced pyknosis, marginalized (vacuolated) chromatin. Bar, 200 nm.
nous nuclease observed that was capable of mediating this transition in the avian erythrocyte.

Osmotic pyknosis from acute ionophore toxicity. Gramicidin is an ionophore that causes salt movement into the cytoplasm and energy consumption. In the avian erythrocyte the effects of the osmotic changes that it, presumably, subjects the nucleus to were observable under phase contrast optics. The nuclei adopted the appearance of being physically compressed, developing distinctive, large, concave indentations of the exterior (Fig. 5) instead of the internal vacuoles characteristic of the other two pyknoses. The concave invaginations were never observed with normal or starvation-pyknosed nuclei and always observed with acute gramicidin toxicity. This effect, strongly reminiscent of deflating beach balls, appeared as a half-mooning in cross-sections of affected cells. Figure 5 shows a section through lysing cells at terminal gramicidin toxicity displaying the concave invaginations as well as the presence of residual internucleofilamental space with an absence of large internal vacuoles. By contrast, true pyknosis as in Fig. 2B (anucleolytic pyknosis) and Fig. 4B (nucleolytic pyknosis) very rarely shows residual internucleofilamental space and is almost always accompanied by distinctive vacuolation (marginalization). The morphological characteristics are those of compression of chromatin, predominantly driven by extranuclear pressure, not a conformation or other structural change.

Induction of the pyknoses with toxins (Table 3). A small but representative sample of cytotoxins gave either no pyknosis or pyknosis without DNA breakage. Antiglutathione reagents induced pyknosis in accord with the expected mode of action of erythrocyte starvation, via glutathione oxidation. However, agents that would be expected to interfere with the major signaling pathways of apoptosis were notably ineffective. Stauroporine, which causes apoptosis in most cell types by interfering with protein kinase signaling [6, 7], had no effect. One ICE inhibitor did not prevent either pyknosis or cytoplasmic death. Camptothecin and etopside, both topoisomerase inhibitors, were without propyknotic effect at concentrations above their usual range of usage. A poly-ADP-ribose polymerase inhibitor was also without pro- or antipyknotic effect. Butyrate, an agent used to inhibit histone deacetylase, had no propyknotic effect in the presence or absence of glucose although it was cytotoxic in the absence of glucose. This latter may be due to it having a weak ionophore effect. As noted, the most unusual and distinctive toxic effects were from gramicidin.

**DISCUSSION**

Pyknosis appears to require some sort of death program distinguishable from simple cellular disintegration because pyknosis only takes place in the stressed but living cells. In this system it also occurred in apparently living cells or, at least, within cells excluding trypan blue and retaining their soluble proteins. In the culture system used in this report, abrupt death by lysis of cells did not cause pyknosis and left the nucleus in a similar state to what it was in when the cell died. Similarly, rapid uncontrolled death from high concentrations of thiol agents (results not shown) or rapid death by butyrate-induced lysis was not pyknotic. The only nuclear changes observed after very rapid deaths were either none at all or a slight nuclear expansion, the opposite to pyknosis. Thus pyknosis induced by slow death by starvation appears to be the result of some slow molecular or metabolic changes associated with the acceptance of death.

However, the starvation death process is not exactly the same as canonical apoptosis as it was much simpler than the apoptosis programs of mammalian cells, the pyknosis showing no evidence of involvement of topoisomerases or nucleases or histone modifications. Death mechanisms this simple may be uncommon but the mechanism or pathway, whatever it is, is unlikely to be peculiar to erythrocytes as the avian erythrocyte nucleus is more likely to be undergoing evolutionary deletion rather than elaboration. Moreover, whatever it is, there is some direct evidence of this simple pathway's existence in mammals [4, 5], probably in coexistence with other pathways.
The work to date has failed to elucidate or even detect the signaling pathways causing starvation pyknosis. None of the three pyknosis types were induced by topoisomerases (I and II) or staurosporine and these cells have no mitochondria to provide a basis for cytochrome c-based events. However, it remains quite possible that specific signaling proteases are involved as only one specific inhibitor was tried.

The chromatin mechanics of pyknosis. Although single, unified explanations of phenomena are always desirable, the avian erythrocyte nucleus has three ways its nuclei can be made to hypercondense. Nucleolytic pyknosis, unknown in these cells in vivo, is readily produced in vitro by nuclease additions, confirming its retention as a molecular option of erythrocyte chromatin mechanics even though the starving erythrocyte showed no evidence of using it despite considerable searching for its presence. Two of the pyknotic processes, starvation anucleolytic and nucleolytic, were characterized by a complete structural transition that left no internucleofilamental space, some of which became a massive vacuole. This conversion of internucleosomal space to vacuole and shrinkage is the familiar “chromatin marginalization” of the literature of programmed death and occurs in both anucleolytic and nucleolytic pyknotoses.

In the case of anucleolytic pyknosis the conformational basis of pyknosis was confirmed by experiments in which the characteristic morphology and density of pyknosis was erased by forcible expansion of nuclei in vitro and on returning the expanded nuclei to normal buffers they remembered their original state, either pyknotic or nonpyknotic as the case was. That is to say whether visibly pyknotic or not, the pyknotic state was a molecularly determined and thus remembered state. The collapse of the chromatin slightly inward but mainly outwards in both nucleolytic and anucleolytic pyknosis to produce a vacuole is a curiously simple but almost unarguable evidence for an interchromatin, intermolecular, contraction force in operation rather than a compression. Contraction driven by nuclear compression would have given a reduced vacuole not a massive new one, and a marginalization driven by vacuolar expansion would have given a distended nucleus as well as a large vacuole not a slightly contracted one. Finally, as noted, there is a negligible nucleoskeleton in these nuclei to complicate this simple, but important argument.

However, the third form of hypercondensation, the from induced by acute gramicidin toxicity, showed a distinctively different substructure from that of the others in the EM section in that considerable dispersed internucleosomal space still remained within the chromatin, and the chromatin’s contraction was expressed as invaginations from the exterior, not as vacuole. Thus both starvation anucleolytic and the nucleolytic collapses had the properties expected from a molecular-level conformational change but the acute gramicidin toxicity type was a contrast in that it mainly had the properties expected from the operation of an external compression force.

As an interesting speculation, it is tempting to postulate that an ionic effect at the nuclear membrane, the opposite to the gramicidin effect reported here, simultaneously with a nucleolytic pyknosis, would explain the appearance of the blebbing (outbulging) of marginalized nuclei often reported as the end stage of apoptosis.

The proposal that the first two pyknotic processes are molecular-level conformational changes strongly suggested that the mechanism of nucleolytic condensation involves internucleosomal rotational states in

<table>
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<tr>
<th>Agent type and condition notes</th>
<th>Toxin name</th>
<th>Effect observed</th>
</tr>
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<tbody>
<tr>
<td>1 (thiol agent)</td>
<td>Glucose starvation</td>
<td>Pyknosis, lytic death</td>
</tr>
<tr>
<td>2 (thiol agent)</td>
<td>Iodoacetate</td>
<td>Pyknosis, lytic death</td>
</tr>
<tr>
<td>3 (thiol agent)</td>
<td>Acrylamide</td>
<td>Pyknosis, lytic death</td>
</tr>
<tr>
<td>4 (protein kinase inhibitor)</td>
<td>Staurosporine</td>
<td>No effect</td>
</tr>
<tr>
<td>5 (topoisomerase I inhibitor)</td>
<td>Camptothecin</td>
<td>No effect</td>
</tr>
<tr>
<td>6 (topoisomerase II inhibitor)</td>
<td>Etopside</td>
<td>No effect</td>
</tr>
<tr>
<td>7, see note for full name</td>
<td>An ICE inhibitor</td>
<td>No effect</td>
</tr>
<tr>
<td>8, PARP inhibitor</td>
<td>Paramino benzamide</td>
<td>No effect</td>
</tr>
<tr>
<td>9, deacetylase inhibitor</td>
<td>Butyrate 4 mm + G</td>
<td>Lytic death without pyknosis</td>
</tr>
<tr>
<td>10, deacetylase inhibitor</td>
<td>Butyrate 4 mm + G</td>
<td>No effect</td>
</tr>
<tr>
<td>11, ionophore</td>
<td>Gramicidin</td>
<td>Complex death (see text)</td>
</tr>
</tbody>
</table>

Note. Cells were incubated in PBS-EDTA, in air (no CO2) at 37°C for 40 h or until lysis, at a density of 1.2 cells per picoliter with 0.2% (+) glucose or without (−) glucose. 1, control cells, starved for 48 h (−) glucose; 1 mM Na iodoacetate. (+) glucose (lysis at 24 h); 3, 2 mM acrylamide (lysis at 72 h); (+) glucose; 4, 2 μM staurosporine (103 h of observation); (+) glucose; 5, 10 μM camptothecin, (−) glucose, and (−) glucose; 72 hours; 6, etopside, 50 μM VP-16, (+) glucose, 48 h; 7, 0.1 μM (IL-1β converting enzyme ICE inhibitor II. ac-tyr-val-ala-ASP-chloromethyl ketone), (−) glucose; 8, 3-amino benzamide, 10 mM (+) and (−) glucose, 48 h; 9, butyrate Na, 4 mM (−) glucose (lysis at 24 h); 10, butyrate Na, 4 mM (−) glucose (no effects at time of lysis of (−) glucose); 11, 4 μg/ml gramicidin-S, (−) glucose, lysis at 2 h.
some way. However, explanations of anucleolytic pyknosis involving ordinary superhelicity were dismissed because the mature avian erythrocyte is known to be rich in topoisomerase I and with almost no [11] or a minimal, temperature-sensitive internal nucleoskeleton [12] to provide antirotation lock points.

In the absence of any agreed structure for the 30 nM fiber of chromatin, a fully defined molecular model is premature, however, there is a testable proposition that currently fits both nucleolytic and anucleolytic pyknosis. It is proposed that both pyknoses are due to a molecular rearrangement in which the “disordered” histone tails of the nucleosomes, that interact with the negative charges of DNA, abruptly shift from predominantly intranucleosomal placing to internucleosomal placings. That is to say the free-energy driving pyknosis is derived from histone tail-sharing entropy. From the evidence in this paper, the most logical candidate for a process that would permit such a general rearrangement of this type would be the noninteger rotational relaxation of internucleosomal rotational angles. This latter would be permitted by generalized nuclelease action that would confer almost full internucleosomal rotations freedom or generalized nucleosomal slippage allowing screwing rotation to rotate nucleosomes with respect to their neighboring nucleosomes to positions of lowest conformational energy. The minimal conformational energy could involve at least two factors when nucleosomal slippage occurs, the base sequence curvature factor known to affect nucleosomal placing energy and the proposed tail-sharing factor. When internucleosomal nucleolysis triggers pyknosis the former process would not be relevant; this suggests that the energy of the pyknotic transition principally arises from the latter source.

Generalized full-integer rotation as catalyzed by topoisomerases cannot permit either process, as 360 degrees of rotation, of course, does not change the relative orientation of adjacent nucleosomes. This explains why endogenous topoisomerase I cannot mediate pyknosis.

As well as the postulated entropy of tail sharing, an additional, potential, source of conformational energy should be noted and that is the potential internucleosomal steric interference energy along the nucleofilament that has been discussed in other articles in other contexts [13]. However, the tail-sharing would seem to be the process most likely to actually cause the increase of nucleosomal proximity of pyknosis.

A solid decision on the relative contribution to the conformational energy from these sources, and perhaps others, is not possible from the types of experiments reported here. A solution to these questions will require the methodology of subnucleosomal-level structural studies.

Finally, whether the hypotheses/explanations offered are correct, this work still demonstrates that very large amounts of chromatin are readily available to study the peculiar conformational state known as pyknosis and the nuclear transition from the living state to that of death.

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