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COMMENTARY
POLYAMINES TO TARGET DRUGS TO DNA

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In neutral solutions polyamines are fully protonated, and hence are really polyammonium cations (PAC). Spermine, for example, carries four positive charges in a linear system, $H_3\overset{+}{N}(CH_2)_3\overset{+}{N}H_2(CH_2)_4\overset{+}{N}H_2(CH_2)_3\overset{+}{N}H_3$. There is a very powerful coulombic interaction between aqueous DNA and such cations, thus the cations are attracted to the DNA over large distances, and once close to the DNA normally remain there for long periods. A key issue is; are the cations mobile, or do they remain at one preferred site for significant periods? The latter is the currently preferred concept, but NMR and EPR evidence will be presented in favour of the former. If the former is correct, then PACs may be able to act as good drug delivery systems. In its simple form the concept is that any drug that acts directly on DNA can be chemically bound to a PAC. Once in the cell, this PAC-drug complex (PAC-D) will be carried to DNA and will move very rapidly along the exposed strands until it recognises the site of action. This may be some special base sequence region, a damaged site, or the PAC-D unit may simply be present prior to potential damage, so that this can be repaired very rapidly. Some of our current studies on these systems are described.

INTRODUCTION

It is not easy to have an original idea in science today, largely because wherever you turn you will find various teams of scientists already way ahead! [It was much easier when there were fewer "builders"]. My original idea, conceived many years ago, was to use "linear" polycations, such as I, based on

$$Me_3\overset{+}{N}(CH_2)_x\overset{+}{N}Me_2(CH_2)_x\overset{+}{N}Me_2(CH_2)_x\overset{+}{N}Me_3 \qquad \text{I}$$

$$(x = 3 \text{ or } 4)$$

tetraalkylammonium cations, to "deliver" molecules or even anions to the vicinity of DNA, without binding tightly. This should occur because, despite its name, DNA is of course a polyanion. These polycations should have very high DNA affinities, but in contrast with ions such as Mg^{2+} or Al^{3+}, or even with neutral intercalators, all of which bind tightly, there is no way in which these "linear" units could become attached to DNA. Thus the idea was to link a series of DNA-active drugs, D, by chemical reaction, to give PAC-D adducts such as II. [This is just one of a very large range of posible structures]. Normally, D is not

$$Me_3\overset{+}{N}(CH_2)_x\overset{+}{N}Me_2(CH_2)_x\overset{+}{N}Me_2(CH_2)_x\overset{+}{N}Me_2 - D \qquad \text{II}$$

attracted to the negatively charged DNA, and on chance encounter, would move away again if no interaction ocurred. However species such as II would be attracted to DNA over considerable distances, once within its negative coulombic field, and would remain close for long periods. However, they should move at diffusion rates

along the DNA, so that D would reach a preferred site very much more efficiently. This would greatly reduce the [D] required, and hence reduce any unwanted toxicity.

We soon realised that Nature already made use of at least part of this concept with the polyamines spermine (III) and its precursors spermidine (IV) and putrescine (V). Because of the name "amine" I failed to appreciate that these species are, in fact, polyammonium cations (PAC) in cellular systems, as depicted in (III–V). [A pH titration curve, using 1 H NMR, is shown in

$$H_3\overset{+}{N}(CH_2)_3\overset{+}{N}H_2(CH_2)_4\overset{+}{N}H_2(CH_2)_3\overset{+}{N}H_3 \qquad \text{III}$$

$$H_3\overset{+}{N}(CH_2)_3\overset{+}{N}H_2(CH_2)_4\overset{+}{N}H_3 \qquad \text{IV}$$

$$H_3\overset{+}{N}(CH_2)_4\overset{+}{N}H_3 \qquad \text{V}$$

Figure 1].[1] So they also have a very high DNA affinity. The major question is, do they avoid strong binding, or do they bridge the phosphate anions via multiple hydrogen-bonds (a process not possible for I or II).

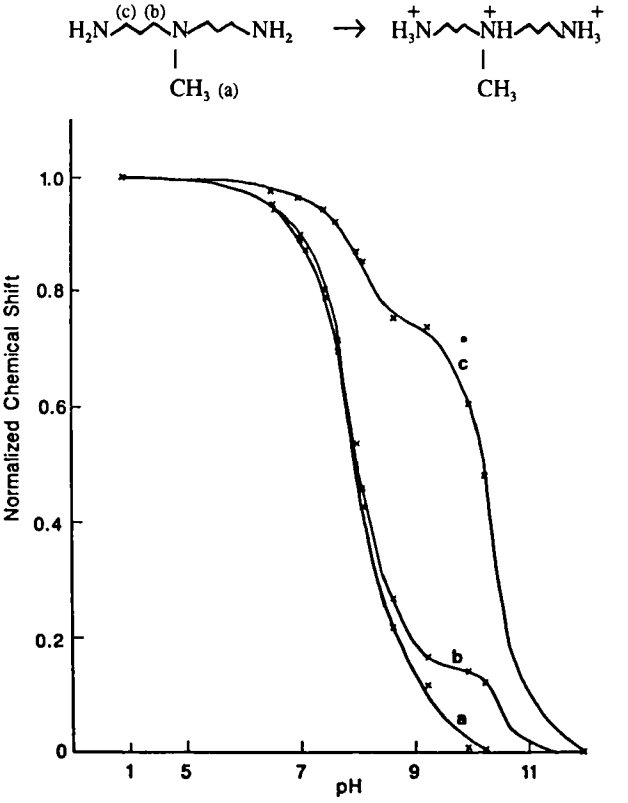


FIGURE 1 Titration curve for the reaction $H_2N\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\underset{\underset{Me}{|}}{N}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}NH_2 + 3H^+ \rightleftharpoons$ $H_3\overset{+}{N}\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\underset{\underset{Me}{|}}{\overset{+}{N}}H\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}\overset{+}{N}H_3$ (c) (b) (a) (b) (c). The curves *a*, *b* and *c* were obtained from the ^{1}H NMR ahifts for the methyl and two pairs of methylene protons. Clearly, the central nitrogen is protonated initially, and there is no distinguishable difference between the two outer amine groups. Protonation is essentially complete at pH 7 (from Ref. 1).

The Role of PACs in Cells

PACs are essential components of all cells. They are normally synthesised within the cell on demand, and are also metabolised within the cell if there is an excess. However there are also active membrane transport systems which help to control their intercellular concentrations. A normal diet provides ample extracellular PACs if there is a need. Their exact role is not clear, but there is strong correlation between intracellular PAC concentrations and cell growth.[2] Whilst the uptake system in most cells is normally closed, that in tumour cells may be more frequently activated because of their rapid proliferation[3] and consequent need for a high concentration of PACs. Some idea of the processes involved within the cell are shown in Figure 2. It seems probable that, because of their polycation nature, their role is to be on DNA, thereby influencing its structure or possibly controlling the interactions between DNA and, say, RNA - but the precise factors involved remain unknown. [I stress that there will also be a strong coulombic interaction with RNA, but have no information on the possible significance of such adducts].

Interaction with Aqueous Duplex DNA

Polyelectrolyte theory requires that the association constants be large, especially for relatively low [PAC] which is the region of interest. This has been fully established in studies using ^{23}Na NMR. Relative to that for dilute aqueous Na^+ ions, the NMR band-widths for ^{23}Na ions near DNA are very large, because of the powerful electric field coupling with the electric moment of the quadrupolar nuclei. For aqueous solutions of the sodium salt of DNA, the Na^+ ions remain quite close to DNA on

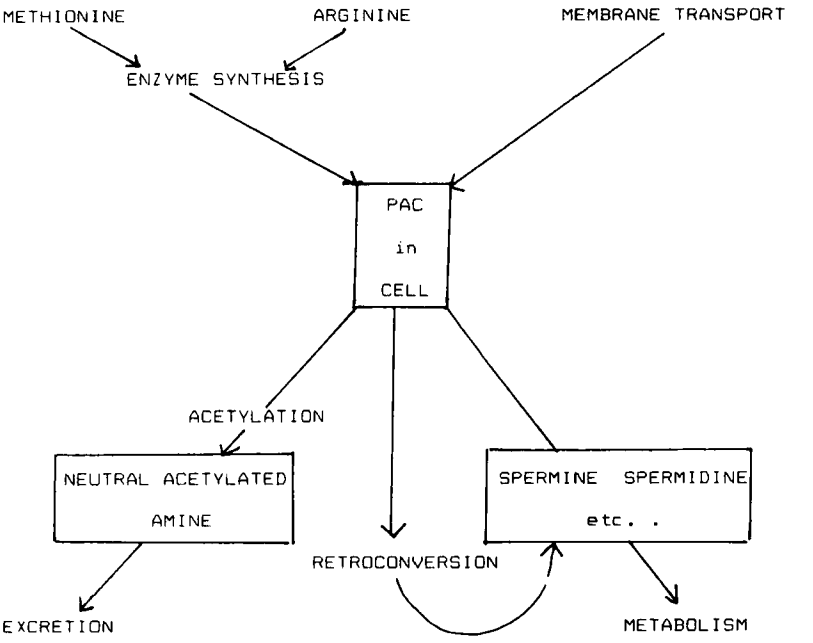


FIGURE 2 Scheme showing the biosynthesis and excretion pathways for cellular polyamines.

average, because of the large negative field, although any individual ion will move in and out of this field frequently and rapidly. Hence fast averaging conditions are observed, the very broad lines for proximal Na^+ change their instantaneous widths to the limiting dilute aqueous width continuously, the observed width being the weighted average of this range. [These changes can equally well be measured using relaxation times]. When PACs are added they flock far more strongly to the DNA, thus displacing those Na^+ ions that are close to DNA in that region. Hence the $^{23}Na^+$ line narrows, and this can be used as a measure of the PAC affinity [Figure 3]. The results confirm that, at low concentrations, this displacement is essentially complete.[1,4]

Mobility of PACs on DNA

There are two opposing schools of thought, one being as implied herein, that the PACs are essentially free to move at normal diffusion rates, spend most of their time close to DNA, but are not hydrogen bonded to DNA for significant periods. The other is that there is strong hydrogen-bonding between the negative oxide ligands of the phosphate groups and the $\delta+$ protons of the ammonium groups. I have tried to represent this pictorially in Figure 4. The spacings between negative and positive charges permit multiple bonding and if one such bond forms, there is a good probability that others will also form. The "chelate effect" will then operate and, for the cation to move away, all of these bonds need to break. Hence the PACs will be immobile for relatively long periods.

It is difficult to predict what should occur. For dilute aqueous solutions of ammonium salts, there is virtually no ion pairing. These cations are strongly solvated by hydrogen bonds to water molecules, as are, for example, $(RO)_2PO_2^-$ ions. Hence

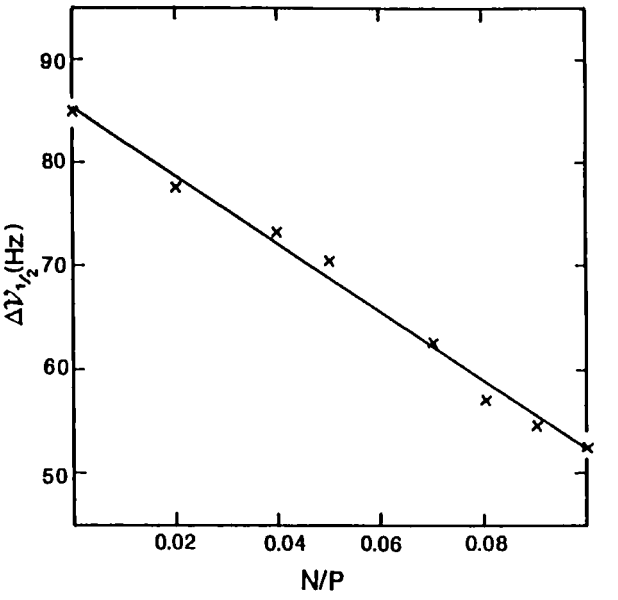


FIGURE 3 Half-widths for the ^{23}Na NMR feature for calf-thymus Na-DNA as a function of added $H_3\overset{+}{N}(CH_2)_3\overset{+}{\underset{\underset{Me}{|}}{N}}H(CH_2)_3\overset{+}{N}H_3$. The relative concentration is expressed as N/P, where N = number of positive charges and P = number of negative charges (from Ref. 1).

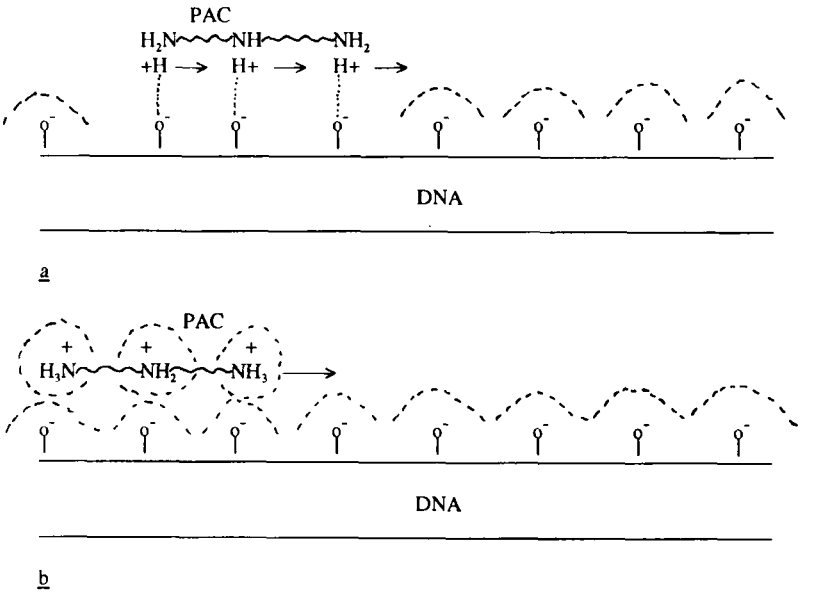
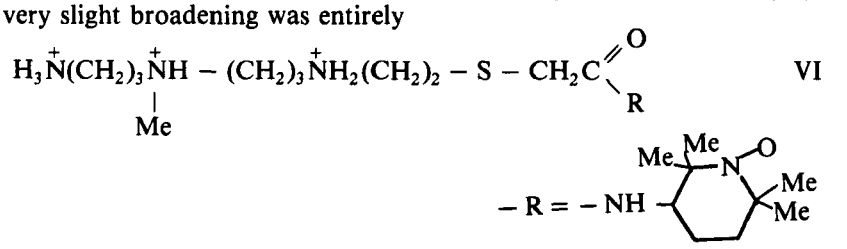


FIGURE 4 *Two dimensional picture for a) "tight" and b) "loose" binding of a PAC to aqueous duplex DNA. In a), there are strong H-bonds holding the PAC to the phosphate ester groups whilst in b) there is only coulombic attraction between the polyanions and cations. [The dashed lines represent solvation by water].*

there are large barriers to contact ion-pair formation. Nevertheless, for DNA, theoretical calculations strongly favour fixed hydrogen bonded sites, bridging across major or minor grooves.[5] Also X-ray crystallographic studies[6] show that such bonding occurs. Nevertheless, I stress that in the latter work, there is no sensible alternative, whilst in the calculations, the number of water molecules used is, perforce, small, and hence the system is effectively extremely concentrated, so that ion-pairing is indeed expected.

Magnetic resonance experiments favour the former concept of essential freedom, with no specific binding.[1,7] For example, the proton resonance features for PAC are only slightly broader in the presence of DNA than in its absence for dilute aqueous solutions. If there was strong binding, the cations would have correlation times similar to that for the DNA, and the lines would be severely broadened. The slight broadening that is observed can be accounted for purely in terms of increased viscosity. More compelling are the EPR results for the spin-labelled PAC (VI). Again, the very slight broadening was entirely



due to increased viscosity. The EPR time-scale requires correlation times of less than 10^{-9} s to explain these results, indicating essentially free motion of the PAC derivative.[1]

In view of these results, and of expectation based on the behaviour of aqueous systems, we have assumed that there is close proximity, but also freedom of motion, when PACs are in solutions containing DNA. Provisional results with cell nuclei and with whole cells suggest that this same freedom of motion occurs for PACs associated with chromatin.[8]

Radiation Protection

This concept of drug delivery to DNA is quite general, but was specifically developed as an aid in radiation-protection.[9,10] Radiation damage leading to cell death does not neccessarily require initial damage to DNA, but nevertheless such damage is bound to occur, and must be repaired if the cell is to continue its proper function. This is, of course, accomplished with exquisite efficiency by cells themselves, but even so, radiation can be both lethal and a major carcinogen, so the development of novel methods of protection is desirable. Our experiments have been aimed at very rapid repair at the redox (electron-transfer) level, the essential idea being to add a redox agent, X, to a PAC, such that X can cycle between X and $X\cdot^-$ (or $X\cdot^+$) thereby picking up excess electrons (or holes) and returning them to give complete reversal of the initial damage (Figure 5).

Our results suggest that PACs themselves do not protect, in normal concentrations,[11,12] although a deficiency may interfere with DNA repair following radiation

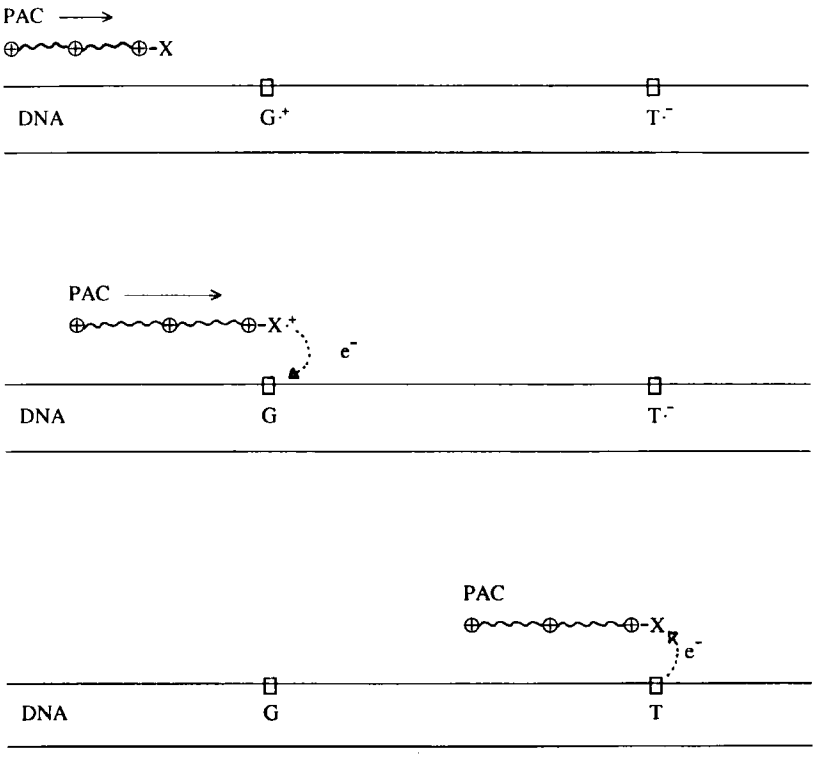


FIGURE 5 Pictorial representation of the mode of action of a redox agent (X) bonded to a PAC and moving close to a DNA strand. This shows electron-donation to "$G\cdot^+$" to give G and $X\cdot^+$, followed by electron-acceptance from "$T^{\cdot-}$" giving T and X.

damage.[13] However, in a major search for any type of protecting agents, it was found sometime ago that the sulphydryl compound (VII) labelled WR1065 is very much more

$$H_3\overset{+}{N} - (CH_2)_3 - \overset{+}{N}H_3 - (CH_2)_2 - S - H \qquad \text{VII}$$

efficient than normal sulphydryl compounds.[13] It is now realised that this compound is present as a di-cation, and the enhanced protection can be understood in terms of the effects described herein.[14,15]

In studies of radiation damage to DNA we and others have used the technique of matrix isolation simply by working at low temperatures.[16–20] Normally, when aqueous solutions are frozen, phase separation occurs to give pure ice together with polycrystalline solute. This is because of the very high effective affinity between water molecules - water loves water. Hence these systems are no longer "solutions" and there is no point to the experiment relative to a study of the solute possibly as a hydrate. However, with polymers such as DNA, although most of the water becomes pure ice, the solvent surrounding the DNA remains in a glassy form, as indicated qualitatively in Figure 6. We find that when we incorporate various compounds into aqueous solutions of DNA, these stay in this glassy region, close to the DNA, on freezing.[21,22] Hence, for frozen aqueous systems, the use of PAC derivatives constitutes no major gain, since they are immobile in the rigid glass.

However, our NMR studies of this glassy water show that "melting" occurs detectably at *ca.* 200 K, and mobility increases to a "fluid" value, but only for the glassy water.[23] At these low temperatures, radical-radical reactions can occur. When an electron-acceptor, (A), is incorporated alone, we observe formation of $A\cdot^-$ and loss of $T\cdot^-/C\cdot^-$ in the DNA. The PAC-A derivative behaves in a similar way, though more efficiently, giving PAC-$A\cdot^-$. On annealing, the A-system has an effect on the decay observed for the radical-cation centres in DNA ($G\cdot^+$). However with the PAC-A derivative, there is a more rapid loss in $G\cdot^+$ and a concomitant loss in the $A\cdot^-$ signal.[8,23] Thus we seem to have observed the hoped-for protection, but only

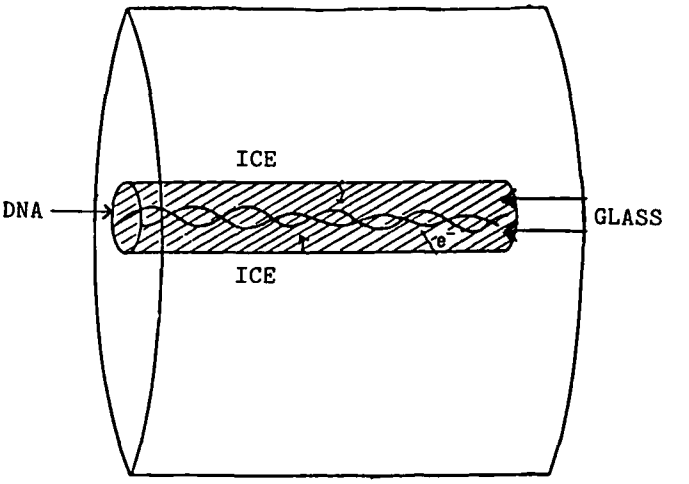


FIGURE 6 Cylinder model for the "glassy" region of water molecules thought to surround DNA in frozen aqueous solution.

on these very artificial systems at low temperatures. Preliminary results with cells, using PAC derivatives, seem to be favourable, but much more work is required.

Radiation-protection induced by PAC-X redox systems or PAC-AH systems probe the hypothesis outlined herein in a clear way, since it requires very rapid movement in order to intercept the damage at the radical stage. Hence, success with these experiments can be taken as good evidence for the general concept.

CONCLUSIONS

I conclude that for any drug systems which act at highly specific sites, incorporation of a PAC unit should greatly increase the efficiency of the drug. On the one hand, the time taken to reach the key site will be greatly reduced, and on the other, far lower concentrations will be needed, and problems associated with general toxicity should be reduced. For drugs that act with DNA indiscriminately, or at units that repeat very frequently, there should still be a gain, though the ability for the PAC system to move rapidly over the DNA is not utilised. The method has a lot of promise, but after several years of study, the sulphydryl derivative (VII) remains the PAC derivative that clearly exhibits increased efficiency induced by the positive charges. However, in this case there is some possible ambiguity in that the proximal positive charge may increase the acidity of the S-H group, hereby increasing the concentration of the anion, RS^-, and hence changing the role from that of hydrogen atom donor to one of electron donor.[23] We hope that studies currently under way will provide us with some more definitive results.

As always in studies on aqueous DNA, there is a big jump to cellular DNA. PACs will presumably flock to any polyanions, including RNA, mitochondrial DNA and nuclear DNA. Considering the last, this is, of course, tightly packaged by looping round positively charged histone proteins, and the resulting strand is itself tightly coiled and organised. Negative phosphate ester groups are still on the surface, and PACs will still be strongly attracted thereto. The extent to which they can penetrate into the inner regions of the chromatin is unknown. If this is restricted, then protection will be largely confined to the outer regions only. These are most exposed to ·OH radical attack whereas the inner regions are most susceptible to direct damage. Furthermore, the degree of compactness of the chromatin varies: the tightly packaged heterochromatin being dormant, and the loosely coiled euchromatin being the active form. The proportion of these two forms varies widely depending on the cell function. Thus, for example, liver cells are particularly rich in euchromatin.

Another aspect of these more complex structures is that if PACs do penetrate into these units, since the local concentration of water is low, they may well bind to the DNA, as in Figure 4b, despite the arguments given above for aqueous DNA! Indeed, such binding may be important in helping to control the degree of "tightness" of these DNA-protein packages.[24]

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