Salk Thursday
23rd Sept 76

Chromatin.

Introduction

Mainly on Structure.
Chromatin: def. DNA, histones, non-histone p. RNA. etc.
The 5 Histones. H1

MW
sequence conservation. H4 } H2A }
H3 } H2B }

molar ratio?
amount per DNA?
will ignore modifications.

The mini-chromosome of SV 40.

circular DNA.
prob. only the 4 histones.
Jack Griffith : diameter ~ 120 Å ——— S
length of DNA (~1:7) (175) ——— S
Its string of beads ——— S
number of beads (~21)
more recent work: Chambon
Yaniv.

ALSO Olins and Olins (Nov 1955)

(2)

~~Xrs~~ Early x-ray studies Pardon + Wilkins. 110, 55, 37, 27 A etc.

interpreted as a supercoil. Bradbury neutron diffraction showed 110 mainly due to protein.

The Breakthrough

Hewish and Burgoyne.

endogenous Ca/Mg endonuclease. (light digestion) on rat: liver nuclei.

DNA pure ~~ox~~, d.s., on acrylamide gels.

bands ~ 200, 400, 600, 800 bp. etc.

(rather broad bands).

① established approx MW

~~should~~ ~~most~~ ② should most DNA in these structures.

Markus Noll (also Ken van Holde)

micrococcal (staphylococcal) nuclease:

~~but~~ Similar result. best estimate 197 ± bp.

showed (heavy) shearing ~~mold~~ muddied the pattern.

~~Hall~~ The 11 S particle ie with histones one.

Sucrose gradient.

The 1. 2. 3, 4 expt. ——————— S

~~Further digestion 140 core particle Felsenfeld (cutting within the particle).~~

The ~~Kor~~ Kornberg model ~~[illegible]~~ ~~[illegible]~~ (histone interactions) Kornberg & Thomas, tetramer $H3_2$ $H4_2$

Amount of DNA : 200 bp ≃ ~~4~~ 2 × 4 histones + H1

Thus the bead (= rhodias ?) = nucleosome)

postulated to have this structure. Size ~ (100 Å)^3 (110 Å)

More recent results

The variation in the 200 bp.

Aspergillus, yeast + neurospora ≈ ~~120~~ 170 bp

~~Pa~~ CHO cells. Lee Compton (Chambon) ~~180 bp~~ 180 bp

Same in interphase as in metaphase

Chick red cells Ron Morris 205. (5 to longer)

~~Chan~~ Sea urchin sperm. 240 bp Chambon

gastrula 220 bp.

Rabbit brain Jean Thomas. ~~neuron~~ neurons 175–180 bp

glia. 195–200 bp.

The 140 bp core particle

'pause' in micrococcal digestion.

So far, same in all ~~particles~~ material studied

i.e. linker varies.

Crystallisation of particle Russian group. Varshavsky + Georgiev.

small : for X-ray powder diagram

Klug + colleagues : small .. e/m picture (~110 Å) — S

larger. ~~sa~~ single crystals : to 20 Å.

(~~Pa~~

Second surprise

DNAse I digestion Markus Noll

fuzzy 200 etc. cuts either particle.

Lopez digest gave 30, 40, 50 ... 80 ... 120 (130) (140) ...

for 55 DNA.

Thus appeared to cut "every" ~ 10 bp (10 bp per turn).

Lex Latter : terminal labelling

~~de~~ assumption of a dyad

10 20 (30) 40 50 (60) 70 (80) 90 100 (110) 120 130 | 140 S

~~cut~~ (or no cuts)

Thus implies cuts are close together on the structure.

Curved or kinked ? turns with no bases compressed.

~~integer argument~~

Crick & Klug kink ——— S.

(Hark) Sobell kink. PNAS ~~[illegible]~~

evidence for kinks : integer argument 80 (not 79 etc).

Number of supercoils : ~~[illegible]~~ concept clarified by Brock Fuller (Cal Tec)

for SV40 (or no H1)

measurements suggest ~ −1¼ per nucleosome.

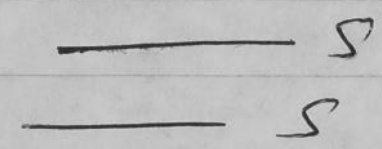
but difficulties about reference state.

meaning : explanatory paper in August PNAS

must distinguish between Twist and Linking number.

Higher order structure

"Solenoids" Finch + Klug. e/m pictures.



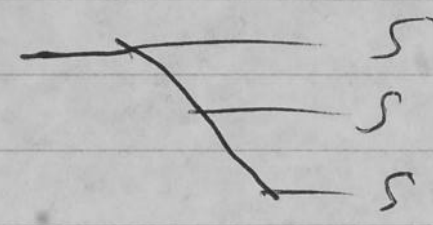
~~[illegible] interpretation~~
Supported by e/m pictures of Howard Davies.
interpretation of X-ray pictures.
~~Bradbury inter~~
Supported by Bradbury's neutron diffraction.

Size of solenoids

Loops

2 groups + worked.

e E. coli (Jack Kuhlth!
~~[illegible]~~ Sm/Tanir protein.



The Future: primary v. tertiary structure.