Effects of the antibiotic netropsin on mouse ascites tumour chromosomes in vitro[1]

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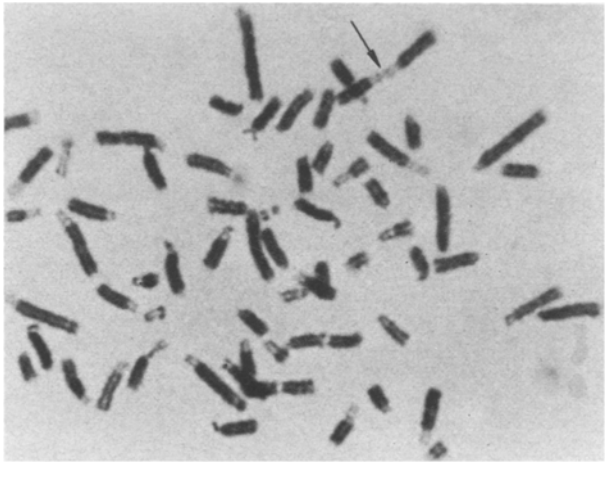
Summary. The antibiotic netropsin was found to induce an increase of the aberration frequency of up to 10% and a decondensation and elongation of centromeric regions of the chromosomes in mouse ascites tumour cells cultivated in vitro.

Netropsin, a highly basic oligopeptide antibiotic isolated from Streptomyces netropsis, specifically binds, in a nonintercalating way, to AT base pairs in double-stranded DNA in vitro[2,3]. Inspite of extensive biochemical and biophysical studies on DNA-netropsin interaction in vitro, present knowledge about the mode of action of netropsin in eukaryotic cells is rather limited[2,3]. We, therefore, looked for specific effects of netropsin on chromosomes of mouse ascites cells cultivated in vitro.

Materials and methods. 5-day-old Ehrlich ascites tumour cells (strain 'Berlin-Buch') were cultivated in vitro in a Warburg apparatus as short-term suspension culture[4,5] at 35 °C for 21 h in the presence of different concentrations of netropsinsulfate (NtS) and netropsinhydrochloride (NtHy), respectively. Colchicine (0.002%) was added 3 h before fixation and the production of air-dried slides. NtHy and NtS were dissolved in sterile Aqua tridest. and the NtS-solution heated for 15 min at 50 °C because of its low solubility. To estimate the aberration frequencies, 50 cells per experiment were analyzed for chromatid breaks (b), chromatid translocations (t) and multiple aberrations (m.a.). Gaps (g) were separately counted but not included in the category of true aberrations; 2×50 cells per concentration of NtS or NtHy were scored with respect for the presence and number of chromosomes showing centromeric elongation. The Ehrlich ascites cell strain used has a stem-line of 44 chromosomes including about one metacentric B-chromosome. The other chromosomes are telocentric.

Results. Netropsin at the highest possible concentration used resulted in an inhibition of cell multiplication and mitotic frequency. Whereas both compounds have about the same cytostatic effect (about 50% inhibition at 10^{-4} M), significantly different percentages of chromosome aberrations and centromeric elongation have been found after treatment with equal concentrations (table). Since NtS and NtHy have exactly the same quantitative binding effects on isolated DNA (C. Zimmer, pers. comm.) different intranuclear concentrations after treatment of intact cells with NtS and NtHy might explain this finding. As shown in the table, netropsin treatment resulted in a low but significant, concentration-dependent increase of the spontaneous aberration frequency. The majority of translocations were found to be located in the centromeric regions. The most pronounced effect of netropsin was the elongation and decondensation of chromosome segments (figure) known to be heterochromatic and to exhibit C-banding[6]. This effect is strongly dose-dependent and specially pronounced in the metacentric B-chromosomes. Heterochromatin of telocentric chromosomes was much less affected since, even at the highest concentrations used (10^{-4} M NtS, 5×10^{-4} M NtHy), only 18–19% reacted with decondensation (table).

Discussion. It is well-known that many compounds acting at the DNA level can lead to chromosome aberrations. This is also true for netropsin as shown in this communication. The most specific and significant effect of netropsin on chromosomes of ascites tumour cells of the mouse is, however, the extension and decondensation of centromeric chromosome regions. At the light microscopic level, similar kinds of segment extension in different chromosome regions are known to be caused by a variety of factors (e.g. Fucik et al.[7] and Hsu et al.[8]); but no unambiguous explanation as to the molecular mechanism underlaying this phenomenon is presently available. Mouse centromeric heterochromatin is known to contain satellite DNA[9,10] with a mean GC-content of 36%, about 5% less than total DNA. However, about half of the light strand contains a short repeat whose original sequence included with high probability $d(GA_5TGA)$[11]. This sequence must be an ideal target for the AT-specific netropsin which covers about 4 AT base pairs[12,13]. Because of its high



Chromosomes of a mouse ascites tumour cell after 21 h of cultivation in the presence of 1×10^{-4} M netropsinsulfate with centromeric elongation (3000 ×). The arrow points to the metacentric B-chromosome.

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Chromatid aberrations and centromeric elongation induced by different concentrations of netropsin sulfate (NtS) and netropsin hydrochloride (NtHy) in Ehrlich ascites tumour cells of the mouse in vitro

Concentration tested (Mol/l) (treatment time 21 h)		Number of metaphases analyzed	Metaphases with aberrations (%)	Types of aberrations per 100 cells				Cells with centromeric elongation (%)	Percentage of metacentric B-chromosomes with centromeric elongation (%)	Percentage of telocentric chromosomes with centromeric elongation (%)
				g	b	t	m.a.			
NtS	$1 \cdot 10^{-4}$	250	9.6	0.8	2.4	2.8	0.4	90.0	100	18.5
	$5 \cdot 10^{-5}$	100	8.0	2.0	6.0	3.0	0	20.0	85.0	2.6
	$2.5 \cdot 10^{-5}$	100	2.0	1.0	2.0	0	0	5.0	60.0	2.1
	$1 \cdot 10^{-5}$	250	2.4	1.2	2.0	0.8	0	2.0	0	2.3
NtHy	$1 \cdot 10^{-3}$	No mitosis								
	$5 \cdot 10^{-4}$	100	4.0	0	0	4.0	0	91.0	100	18.7
	$1 \cdot 10^{-4}$	300	4.0	2.0	2.7	2.3	0	33.0	87.9	2.3
	$7.5 \cdot 10^{-5}$	100	2.0	4.0	2.0	0	0	18.5	67.6	2.6
	$5 \cdot 10^{-5}$	100	2.0	2.0	2.0	0	0	13.0	69.2	2.1
	$1 \cdot 10^{-5}$	200	2.5	0.5	2.5	1.0	0	Not determined		
Control		300	0	0.7	0	0	0	0	0	0

binding constant, netropsin should be able to remove proteins thought to be responsible for chromatin compactness, especially histon H1. This effect was indeed demonstrated in experiments with calf chromatin[14] and could be a molecular reason for the heterochromatin decondensation observed. Netropsin binding to other than satellite DNA can be inferred from the observation that netropsin-treated chromosomes stain much less than untreated chromosomes with aceto-orcein. In this respect it is important to note that the bibenzimidazol derivative 'Hoechst 33258', another highly AT-specific compound which causes centromer uncoiling in some mouse cell lines[15,16] but not in human chromosomes[16], is without detectable effect on ascites chromosomes of cells grown in a permanent or the short-term suspension culture described here under the conditions used by Kim and Grzeschik[16] (unpublished results).

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Neural inducing capacity of cyclic AMP on post-nodal pieces of early chick blastoderms[1]

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Summary. Cyclic AMP (0.5 mM) induced neural differentiation in post-nodal pieces of early chick blastoderms, at least in part, through promoting cell movement, tissue condensation, and assembly of microtubules.

Hensen's node has long been recognized as the center for the primary organization of the chick embryo. Pieces isolated from regions more than 0.5 mm posterior to the node, regardless of culture techniques and duration of cultivation, do not develop well-defined axial structures[2-10]. Thus post-nodal peices (PNPs) have frequently been used for testing the inducing capacity of various agents.

Experiments previously conducted in our laboratory[11] showed that cyclic AMP and several related nucleotides could induce differentiation in PNPs. The inducing capacity of these nucleotides varied and was concentration dependent. Differentiation was usually manifest by the formation of neural tissue, but concentrations used were unphysiologically high (3–18 mM) and many PNP cells showed signs of mild cytolysis. Thus a question was raised whether the observed differentiation was a consequence of cyclic AMP treatment or sublethal damage. Somewhat different results were recently reported by Deshpande and Siddiqui[10] who found that cyclic AMP, at a lower concentration (0.5 mM), induced the formation of heart-like pulsatile tissues in 74% of the PNPs. The present study was undertaken to reinvestigate the inducing capacity of cyclic AMP on PNPs.

Materials and methods. Unincubated fertile White Leghorn eggs were obtained from the Shamrock Poultry and

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