fresh water bivales are probably derived from marine forms.

Of the species studied here and by other authors[1,2], octopine dehydrogenase was absent from the opisthobranchs, whilst in the prosobranchs the enzyme occurred widely in the Archaeogastropoda and Neogastropoda but has rarely been reported from the Mesogastropoda. The pulmonates and the opisthobranchs are thought to have split off early in evolution from the prosobranch line, possibly from the mesogastropod stock[7]. Whether this correlation between the distribution of octopine dehydrogenase and the taxonomy of the gastropods is correct will require data from many more species. Interestingly the primitive polyplacophoran *Lepidochitona cinerareus* showed no octopine dehydrogenase activity, only lactate dehydrogenase being detected (290 nmoles/min/mg protein). These results suggest that octopine dehydrogenase may have arisen on more than one occasion during the evolution of the Mollusca.

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Incorporation of base analogues for detection of unusual base compositions in plant metaphase chromosomes (*Vicia faba*, Leguminosae)

I. Schubert, R. Rieger and P. Döbel[1]

Zentralinstitut für Genetik und Kulturpflanzenforschung der Akademie der Wissenschaften der DDR, DDR-4325 Gatersleben (German Democratic Republic), 15 December 1980

Summary. Bromodeoxyuridine and bromodeoxycytidine with fluorescence-plus-Giemsa staining resulted in similar patterns of asymmetric bands in *Vicia faba* chromosomes. Limitations as to the use of methods for identification of A + T- and G + C-rich regions are discussed.

During the last years many attempts have been made to visualize, at the microscopic level of resolution, metaphase chromosome regions with unusual base composition. One method for the detection of A + T- or G + C-rich regions is the comparison of banding patterns induced by base-specific fluorochromes, which in vitro show preferential affinity for A + T- or G + C-rich DNA sequences[2-6]. Another method is in situ hybridization with radioactively labelled homopolynucleotide sequences. A 3rd, and perhaps more direct method is the autoradiographic detection of incorporated radioactively-labelled DNA precursors. The power of resolution of this method has been improved by introducing differential staining techniques for the demonstration of halogenated nucleoside analogues (e.g., by means of the fluorescence-plus-Giemsa technique[7]).

A pulse treatment with either bromodeoxyuridine (BrdU), bromodeoxycytidine (BrdC) or azacytidine (AzaC) during the end of S-phase results in pale bands after Giemsa-staining, fluorescence-plus-Giemsa staining (BrdU, BrdC) or Feulgen staining (AzaC) of metaphase chromosomes of man[8-10] and *Vicia faba*[11,12]. These bands seemed to be identically positioned (fig. 1) and are therefore interpreted by most authors as representing late replicating DNA rather than as being the result of base-specific incorporation of the analogues in these regions, which, after appropriate pretreatment, in most cases show up with dark Giemsa bands (fig. 1). This interpretation gains additional support from autoradiographic labelling of the same chromosome regions after pulse treatment with ^{3}H-thymidine during late S-phase[12].

In *V. faba*[13] (as in animals: man, mouse, *Cricetulus triton, Dipodomys ordii,* and *Drosophila nasuta*), incubation of cells for 1 cell-cycle in BrdU just prior to fixation resulted in asymmetric dark bands after application of a fluorescence-plus-Giemsa technique. This banding pattern has generally been interpreted as giving evidence of regions with uneven distribution of adenine and thymidine between the 2 strands of the DNA double helix. Therefore it was of interest to study the effects of treatment with BrdC or AzaC instead of BrdU. Unfortunately, AzaC did not result in chromosome differentiation after treatment for 1 or 2 cell cycles, neither after Feulgen staining nor by the use of the fluorescence-plus-Giemsa technique. Probably, the mechanism leading to AzaC banding subsequent to pulse treatment and Feulgen staining is quite different (AzaC inhibits normal methylation of the cytidine moiety[14,15]) from that giving rise to corresponding bands after

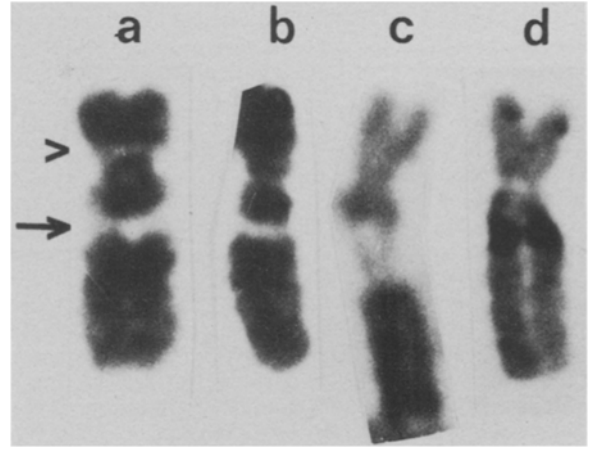

Figure 1. A sample of 4 chromosomes V of the reconstructed *Vicia faba* karyotype ACB (with a pericentric inversion in standard chromosome V). *a* DNA late replication bands (arrows) after a short pulse with BrdU and subsequent fluorescence-plus-Giemsa staining[12]; *b* the same after incubation in BrdC; *c* 'segment extension' after pulse treatment with AzaC and subsequent Feulgen staining[11]; *d* Giemsa-bands of the same chromosome[12].

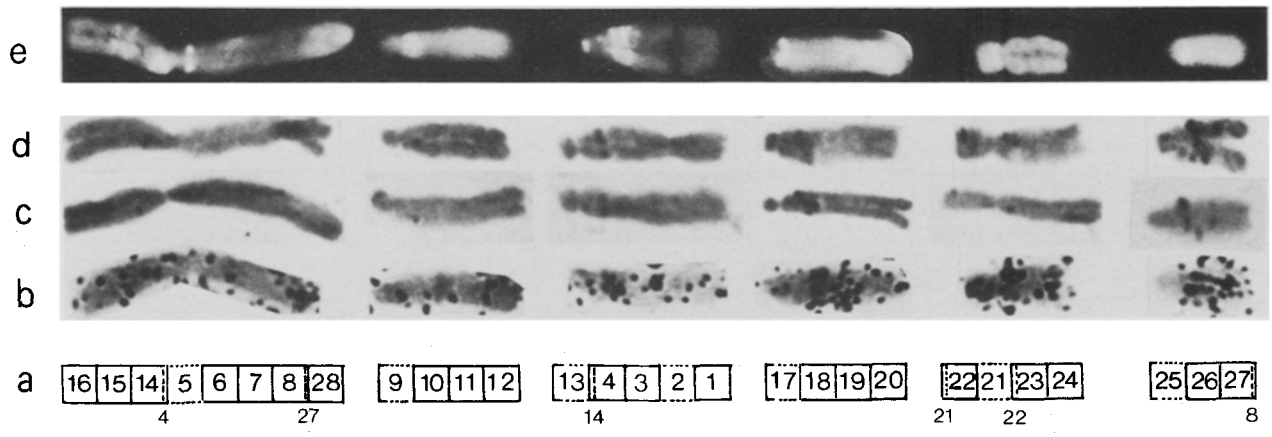

Figure 2. Schematic subdivision of the multiply reconstructed *Vicia faba* karyotype ACB. *a* The sequence of segment numbers demonstrates the positional changes of chromosome segments as compared to the standard karyotype; *b* A chromosome complement showing silver grains after in situ hybridization with ^{3}H polyuridylic acid (sp. act. 4.65 Ci/mM UMP, 90,000 cpm per slide, hybridized 15 h in 2× SSC at 24 °C)[22]; *c* A chromosome complement showing asymmetric dark bands after incubation of cells in BrdU for 1 round of DNA replication and subsequent fluorescence-plus-Giemsa staining[13]; *d* the same after incubation for 1 cell cycle in BrdC (10^{-4} M), but without FUdR and uridine in the medium; *e* A chromosome complement with Q-banding after quinacrine dihydrochlorid treatment[12]. Notice that only the 2 Q$^+$-bands of segment 4 (chromosome III) seem to be positionally coincident with asymmetric bands (see *c* and *d*).

BrdU or BrdC treatment and fluorescence-plus-Giemsa staining[16].

BrdC, however, gave similar results to BrdU incorporation; sister-chromatid exchanges after 2 rounds of replication and asymmetric bands (fig. 2) after 1 round of replication and subsequent fluorescence-plus-Giemsa staining.

BrdC-induced asymmetric bands are less clear than those induced by BrdU; otherwise they fulfil the same criteria as the latter[13]. This might mean that the chromosome regions characterized by an uneven distribution of A and T between the DNA strands at the same time contain an uneven distribution of G and C. In fact another explanation seems to be more plausible; the greater part of the BrdC becomes cellularly metabolized to BrdU[17] and is thus incorporated into chromosomal DNA instead of thymidine. A similar conclusion was drawn by Zakharov and Bairamjan[18] who compared sister-chromatid exchange induction by means of BrdC in human lymphocytes with and without excess of thymidine in the culture medium.

The data presently available thus exclude an unambiguous demonstration of the presence of chromosome regions with a special composition of G + C, both after incorporation of AzaC or BrdC and by comparison of the patterns of distribution of chromatid aberrations induced by ^{3}H-C or ^{3}H-T[19].

The chromosome regions marked by asymmetric bands after 1 round of replication in BrdU do not seem to be the only regions of polydA · dT (if they do represent such regions) since in situ hybridization with ^{3}H polyuridylic acid resulted in a distribution of silver grains much more unspecific than the distribution of asymmetric bands (fig. 2). This is true even when the more limited power of resolution of autoradiography is taken into account, since in most cases the whole chromosome set appeared to be evenly labelled. The same situation was found in Chinese hamster cells[20] and in *Chironomus thummi* polytene chromosomes (Schubert and Serfling, unpublished) and may be an indication of the presence of short polydA · dT sequences dispersed throughout the genome.

The asymmetric bands have, on the other hand, been found in only 2 out of 14 cases to be positionally coincident with Q$^+$-bands obtained by staining with quinacrine dihydrochloride (fig. 2). These Q$^+$-bands are taken to demonstrate the presence of A + T-rich chromosome regions[2]. The weak positional coincidence of asymmetric and Q$^+$-bands may mean that either the Q$^+$-bands do not in fact demonstrate A + T-richness[21] or that asymmetric bands are indicative of an uneven distribution of adenine and thymidine between chromosomal DNA strands, independent of the relative A + T content of the regions in question.

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