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A concerted polymerization-mesophase separation model for formation of trichocyte intermediate filaments and macrofibril templates. 1: Relating phase separation to structural development

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ABSTRACT

The concept that macrofibril templates, the fibrillar precursor to complete macrofibrils incorporating matrix proteins in trichokeratins, are formed by intracellular anisotropic phase separation of intermediate filaments (IFs), is here developed in detail. The factors affecting structural development, including IF length dispersion, and presence of other macromolecular solutes, are discussed in terms of the statistical thermodynamic models presented over 30 years ago by P.J. Flory and co-workers. The crucial role of pendant IF head groups in controlling IF separation and stabilizing the mesophase is emphasised. In particular, a concerted process of polymerization of unit-length IF precursors coupled with continuous transfer of longer IFs to the anisotropic phase is invoked. Observed structures in differentiated cell lines in mature fibres are rationalised in terms of different possible nematic or double-twist liquid crystalline precursor structures, with varying degrees of anisotropic phase coalescence. The occurrence of rarely observed macromolecular double-twist structures is made plausible by qualitative analysis of mesophase mechanics and reference to alternative structures in other macromolecular mesogens. The model is consistent with, and explains, certain well-known features of mature fibre structure, such as filament-matrix ratios in different cell lines. A few comments relating to the infill of the template by keratin intermediate filament associated proteins (IFAPs) are presented.

1. Introduction

One of the authors has previously presented a basic theory (McKinnon, 2006) that macrofibril formation within the cortex cells (trichocytes) of developing animal fibres comes about initially through separation of an intracellular mesophase created from highly anisometric (rod-like) intermediate filaments (IFs) in the cytoplasm. During later development within the follicle these proto-macrofibrillar structures (here called macrofibril templates) formed in the cytoplasm are infiltrated with associated proteins (IFAPs), which, during subsequent keratinisation, are covalently bonded to one another and to the IFs, and dehydrated to form the finished macrofibrils as observed in the cortex of mammalian hairs. It was postulated (McKinnon, 2006) that prior to IFAP in-fill and keratinisation the mesophase is stabilised at a given rod–rod separation by the conformational entropy of the pendant intermediate-filament protein head groups (which have some properties peculiar to this class of IF proteins) though it cannot be ruled out that IF tails may also contribute in such a role.

The model was justified by TEM evidence (McKinnon, 2006; Orwin, 1979; French and Hewish, 1986; Woods and Orwin, 1982; Marshall et al., 1991; Morioka, 2005) that proto-macrofibrillar structures appear very early, not just close to cell membranes and associated with desmosomes, but as small, oriented, and relatively concentrated structures isolated in the cytoplasm (Fig. 1). These proto-macrofibrils have a circular transverse profile and first appear just above the bulb (~150 μm from the base) in follicle zones C and D, as defined by Orwin (1979), during the early stages of keratin intermediate filament protein (KIFP) expression. They exhibit all the characteristics of tactoid formation (Bernal and Fankuchen, 1941) by a low-volume-fraction anisotropic phase. "Tactoid" here refers to an anisotropic emulsion droplet, created in the first stage of separation of one liquid phase from another, such droplets being anisometric (often spindle-shaped) on account of anisotropic surface tension arising from inclusion of anisometric

Abbreviations: χ, Flory–Huggins solvent interaction parameter; HGT, high glycine–tyrosine; IF, intermediate filament; IFAP, intermediate filament associated protein; KIFP, keratin intermediate filament protein; ULF, unit length filament.

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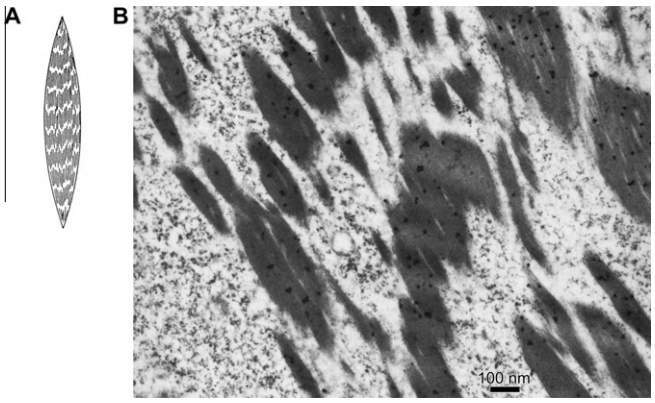

Fig.1. (A) Diagram of a tobacco mosaic virus tactoid (Bernal and Fankuchen, 1941) (© J.D. Bernal, I. Fankuchen, 1941, originally published in J. Gen. Physiol. 25, 111–164). Note that while nematic-type IF tactoids will have the same overall shape, the length dispersity of IF filaments will be much greater, so that the structure internally will not have the smectic-like layers shown in this TMV diagram. (B) An oblique longitudinal section (French and Hewish, 1986) through an array of IF tactoids in a wool follicle, showing their tactoidal shape (© P.W. French, D.R. Hewish, 1986, originally published in J. Cell Biol. 102, 1412–1418). This micrograph depicts an ultra-thin section (ca. 50–80 nm) through the tactoid array, and electron-dense particles on tactoids are from immunolabelling for keratin proteins.

molecules or particles. Tactoids thus signal the emergence of an oriented liquid crystalline phase (mesophase) from an isotropic solution. With increasing volume fraction of anisotropic phase, tactoids coalesce into elongated macrofibril templates (Figs. 2 and 3), as is found in other cases of unidirectionally extended macromolecular mesophases (Bernal and Fankuchen, 1941; Gulik-Krzywicki et al., 1993). The association of the observed structural motifs with liquid crystal mesophases, of both untwisted (nematic – perhaps, more correctly, twistless cholesteric) or double-twist character is portrayed in Fig. 4, which compares the fibrillar structures observed in different cortical cell types by electron tomography (Caldwell et al., 2005) with the idealised structures of mesophases (de Gennes and Prost, 1993; Crooker, 2001; Livolant, 1991).

Here we build on the earlier work (McKinnon, 2006) especially by coupling the phase separation process to an IF formation process, in which IFs are formed by the one-dimensional aggregation (elsewhere called an "annealing" and here a "polymerization") of short full-width (nominally 32-chain) unit length filaments (ULFs), in a concerted process of "frustrated" equilibrium polymerization and phase separation. Such a model of biomolecular self-assembly was proposed over 30 years ago by Flory and Frost (1978), but this model has apparently never been hitherto invoked to explain an *in vivo* self-assembly process. We add the descriptor "frustrated" here because the continuous transfer of longer members of the polymer distribution to a separate anisotropic phase results in the polymer distribution never attaining equilibrium; concerted polymerization and phase transfer is thus a non-equilibrium process, which may be entirely consistent with a key event in such a biological environment.

Such theories, either of phase separation or of polymerization, are not confined in principle to any particular size of the mesogen or monomer unit, although some of the details may be size specific; here the "monomer" (ULF) is a very large macromolecular complex of some 1.6 MDa. There are plenty of precedents for the lyotropic phase separation of such large mesogens, a classic example being tobacco mosaic virus (Bernal and Fankuchen, 1941, Fig. 1A); the most closely relevant example in size terms is perhaps the family of fd viruses (Dogic and Fraden, 2000, 2001; Lapointe and Marvin, 1973). Examples of hydrogen-bonded macromolecules forming mesophases and fibrils analogous to those proposed here are to be found in supramolecular synthetic chemistry (Gulik-Krzywicki et al., 1993; Bonnazzi et al., 1991).

In this paper, Part 1, we concentrate on discussing the basic filament formation and phase separation processes, and relate them to various well-known structural features of animal fibre cortical cells.

In a proposed follow-up paper (Part 2), the further step will be taken, based largely on the observed rapid and cell-specific proliferation of IF tactoids and filaments, of postulating that the aggregation of ULFs into macrofibril templates is a sudden-transition event (effectively a ceiling temperature phenomenon) which can be

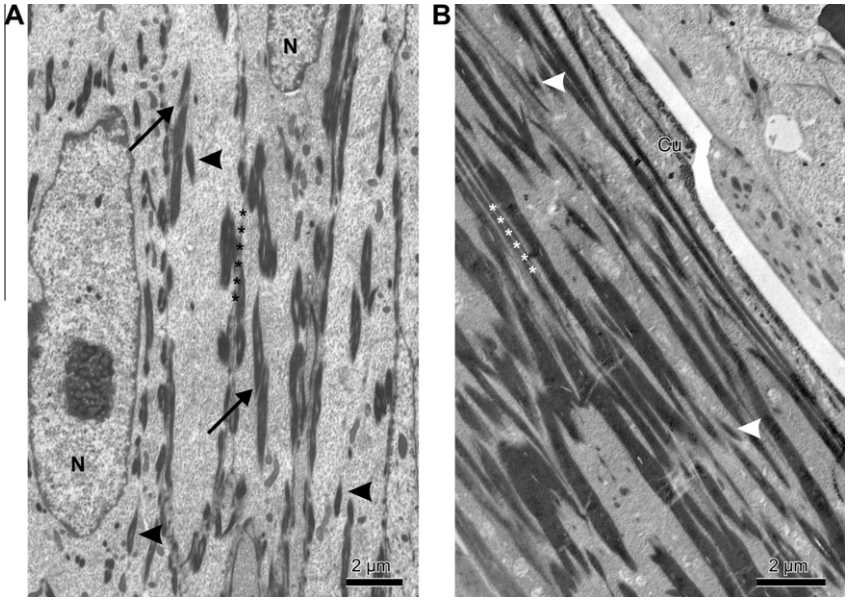

Fig. 2. (A) Longitudinal section through wool follicle cell in low zone C (Orwin, 1979), showing predominantly nematic tactoid development and coalescence (right hand side). Arrows mark instances of two tactoids deforming in the process of partial or complete coalescence; each tactoid is an aggregate of several smaller ones. (Reproduced as a detail from Woods and Orwin (1982), copyright 1982, with permission from Elsevier.) (B) Another longitudinal section, of zone D, showing well-formed nematic-type (paracortical) tactoids, and coalescence in some cases into extended macrofibril templates. (Micrograph courtesy of J.L. Woods, from AgResearch archives.) In each micrograph: arrowheads indicate examples of small well-formed tactoids, about 700 nm length; N = nucleus; Cu = developing cuticle cell; ∗ = example of cell membrane.