Molecular mechanism of bacterial type 1 and P pilis assembly

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The formation of adhesive surface structures called pilis or fimbriae (‘bacterial hair’) is an important contributor towards bacterial pathogenicity and persistence. To fight often chronic or recurrent bacterial infections such as urinary tract infections, it is necessary to understand the molecular mechanism of the nanomachines assembling such pilis. Here, we focus on the so far best-known pilus assembly machinery: the chaperone–usher pathway producing the type 1 and P pilis, and highlight the most recently acquired structural knowledge. First, we describe the subunits’ structure and the molecular role of the periplasmic chaperone. Second, we focus on the outer-membrane usher structure and the catalytic mechanism of usher-mediated pilus biogenesis. Finally, we describe how the detailed understanding of the chaperone–usher pathway at a molecular level has paved the way for the design of a new generation of bacterial inhibitors called ‘pilicides’.

1. Introduction

Bacterial pilis or fimbriae (used here as synonyms), meaning ‘hairs’ or ‘threads’ respectively, were first described in the 1950s [1]. These surface structures made of thousands of protein subunits (called pilins) are known to be essential for biofilm formation [2], and are particularly relevant to human health, because the tip proteins of such pilis, called adhesins, are able to bind to specific host tissues. This adhesion step is crucial in the host colonization cycle by both Gram-positive and -negative bacterial pathogens (for review, refer to [3–5]).

Uropathogenic Escherichia coli (UPEC) strains encode up to 13 distinct pilus-encoding gene clusters, including
2. Pilus morphology, function and subunits

(a) Morphology

The minimum components of a CU pilus are a major pilin subunit, a periplasmic chaperone, an outer-membrane (OM) pore/pilus assembly platform termed usher and, in most cases, a tip adhesin. All CU pathway components in Gram-negative bacteria are initially translocated through the inner membrane (IM) via the Sec machinery [8]. The usher—FimD and PapC for the more extensively studied type 1 and P pili which are assembled via the classical chaperone–usher (CU) pathway. The F1 pilus are produced by Yersinia pestis via the Caf1 CU pathway, and the CFA/I pilus are assembled in enterotoxigenic E. coli (ETEC) via the alternate CU pathway (figure 1). Besides the CU pathways, there are two other pili assembly pathways: (i) the curli biogenesis or nucleation/precipitation pathway and (ii) the type IV pilus assembly pathway (for details, please see reviews [6,7]). In this review, we essentially focus on structural, mechanistic and kinetic insights gained on the type 1 (Fim) and P (Pap) pili, which are mainly found in UPEC strains and are mostly involved in the development of highly recurrent urinary tract infections (UTIs) such as cystitis (type 1 pilus) or pyelonephritis (P pilus). These infections affect around 40% of women and around 12% of men at least once during their lifetime [3]; UTIs are the second most common infectious diseases category worldwide.
type 1 and P pili, respectively—is embedded into the OM via the β-barrel assembly machinery (BAM complex) [9,10]. The periplasmic chaperones FimC and PapD promote subunit/pilin folding as they are released by the Sec translocon and target pilus subunits to their respective usher [11,12].

Several distinct morphologies for pili assembled by the CU pathway have been described: (i) the F1 pilus which is a flexible and thin fibre (approx. 2 nm in diameter) [13]; (ii) type 1 or P pili made of a right-handed helical rod of thousands of major pilin subunits, FimA and PapA, respectively (approx. 6–8 nm in diameter, approx. 1–3 μm long), linked to a short (approx. 15 nm for type 1 pili and approx. 40 nm for P pili) flexible tip fibrillum made of several different subunits (see figure 1 for details) [14–16]; and (iii) the CFA/I or CS1 pili with helical arrangement of the main pilus subunits CfaB or CooA, respectively, and no distinct tip fibrillum apart from a single minor pilin/adhesin at its distal end, CfaE and CooD, respectively [17,18] (figure 1).

(b) Adhesin function

The main role of the CU pili is adhesion to host cells. Adhesion is mediated by the tip subunit, the adhesin, which in contrast to the other pilus components has two domains: one C-terminal structural pilin domain and one N-terminal receptor-binding/lectin domain that specifically recognizes carbohydrates of the host’s epithelium. In P pili, the adhesin is PapG and its lectin domain binds the Galα–1,4-Galβ moieties present in the globoseries of glycolipids on epithelial kidney cells. The adhesin of type 1 pili is FimH and it binds to the D-mannosylated proteins of epithelial bladder and kidney cells [19]. Generally, several CU pathway gene clusters can be found within one specific strain, a fact probably accounting for the need for several receptors at the different stages of infection until the final target tissue is colonized [19,20].

Several biomechanical studies on pili have shown that there is a certain flexibility in the helical arrangement of the pilus rod which allows it to withstand mechanical stress such as high flow forces in the urethra, while remaining attached to the host [17,18,21–24]. In addition, when the connection between the two FimH domains is exposed to tensile forces, the lectin domain of the tip adhesin is able to undergo drastic allosteric changes from a low- to a high-affinity state to clamp around the mannose receptor [25,26]. The recent FimD–FimC–FimF–FimG–FimH structure shows that the emerging FimH is in its relaxed/open clamp/low-affinity state ready for receptor binding [27].

(c) Subunit structure and molecular mechanism of the chaperone

The chaperone is a 25 kDa protein consisting of two Ig-like domains arranged in a boomerang shape. The pilus subunits, when released into the periplasm, form a disulfide bond between their β-strands A and B (figure 2), a process catalysed by the periplasmic oxidoreductase DsbA [28,29]. It has been shown that the chaperone is able to discriminate subunits with a correct disulfide bond, offering an early stage of subunit quality control [30]. However, even with a correct disulfide bond, pilins are still intrinsically unstable because they have an incomplete immunoglobulin (Ig)-like fold that lacks its seventh, C-terminal β-strand, resulting in a deep hydrophobic groove on the subunit’s surface. The pilin is stable only when the cognate chaperone completes the pilin’s Ig-like fold by occupying the pilin’s groove with part of its own G1 strand, a process called donor strand complementation (DSC) [31–33] (figure 2). DSC stabilizes pilus subunits as they emerge from the Sec translocon in the IM, promotes their folding, and also prevents their premature self-polymerization in the periplasm [12,34]. During DSC, the chaperone’s G1 strand runs parallel to strand F of the pilus subunit, and, thus, the non-covalently assembled Ig-fold that results is non-canonical. The G1 strand is characterized by a conserved motif of four alternating bulky and hydrophobic residues (termed ‘P1 to P4’ residues), which insert into corresponding regions (termed ‘P1 to P4 pockets’) of the receiving subunit’s groove. This chaperone–subunit complex targets the subunit to the usher where it is incorporated into the growing fibre.
Figure 2. Molecular details of the pilus polymerization mechanism. Donor strand complementation (DSC) and donor strand exchange (DSE) mechanisms are shown schematically as topology diagrams (above) and through crystal structures (below, based on PDB coordinate 3JWN). The conserved disulfide bond (S–S, in green) between the β-strands A and B of the subunit (in red) is indicated. The chaperone (yellow) donates one of its strands (G1 strand) to complete the subunit’s Ig-like fold (red), occupying the subunit pockets P1–P4 but leaving the P5 pocket free. During subunit polymerization, the N-terminal extension (Nte) of the next (incoming) subunit in assembly (blue) replaces progressively the chaperone’s G1 strand in a ‘zip-in–zip-out’ manner, starting with the insertion of the P5 residue of the incoming subunit’s Nte into the P5 pocket of the receiving groove, and progressively invading the receiving groove to occupy the pockets P4 to P2. (Online version in colour.)

(d) Subunit–subunit assembly

Every subunit, with the exception of the tip adhesins FimH and PapG, has an N-terminal extension (Nte) of 10–18 residues. Subunit–subunit interaction occurs via a process termed donor strand exchange (DSE) [32] (figure 2): in a chaperone–subunit complex, the chaperone’s G1 strand is inserted into the groove of the subunit it complements (also termed the ‘receiving’ groove); this G1 strand is substituted with the Nte of the subunit next in assembly. Insertion of the Nte is, in this case, antiparallel to the F strand of the complemented subunit, and, thus, a canonical Ig-fold is reconstituted, resulting in an energetically more favourable state that drives the DSE process [13]. These Ntes contain five alternating hydrophobic residues (also termed ‘P1–P5’ residues), which the receiving subunit’s groove accommodates in its corresponding groove’s P1–P5 pockets. The P5 pocket in chaperone–subunit complexes is empty, because the G1 strand of the chaperone only occupies the P1–P4 pockets. The P5 pocket is where the incoming subunit’s Nte initiates DSE, which proceeds via a gradual displacement of the chaperone’s G1 strand by the subunit’s Nte in a ‘zip-in–zip-out’ mechanism (figure 2) [13,35,36]. Once the Nte of the next subunit has zipped into the groove of the preceding subunit, the chaperone is released, and the next cycle of DSE with another chaperone–subunit complex can follow.

It is yet to be established how the length of type I and F1 pili is regulated. However, in the case of the P pilus, a terminator subunit (PapH) could be identified, which lacks a P5 pocket and therefore is unable to undergo DSE, thereby ending the pilus extension process [37,38].
3. Subunit ordering and pilus elongation: the usher’s role

The usher has five structural domains (figures 1 and 3): an N-terminal periplasmic domain (NTD, approx. 125 residues), a 24-stranded β-barrel/translocation pore domain (approx. 500 residues) embedded in the OM and interrupted only by a plug domain (approx. 110 residues), and two C-terminal periplasmic domains (CTD1, CTD2, together approx. 170 residues) [39–41]. The structure of the translocation pore of PapC shows an usher occluded by the plug domain and the dimensions shown in figure 3 [42]. This is the inactive or apo form of the usher, preventing passage of solutes or periplasmic proteins. In its active form, the translocation pore transits from an elongated kidney shape to a more round conformation (figure 3) and the plug domain swings out of the pore lumen into the periplasm close to the NTD to allow translocation of the pilus subunits. This transition from an closed/inactive to an open/active state of the usher is triggered by the recruitment of the first chaperone–subunit complex to the usher (the chaperone–adhesin complexes FimC–FimH in type 1 pili and PapD–PapG in P pili) [43]. Exactly how this recruitment induces pore opening or other conformational changes affecting the various usher domains is still unclear.

(a) The N-terminal domain’s role: subunit recruitment

The usher N-terminal domain (NTD) is the initial recruitment site of chaperone–subunit complexes, a fact confirmed when it was observed that a bulky molecule reacted to the NTD could inhibit pilus elongation in vitro [43–45]. In addition, the NTD alone has by far the highest affinity for chaperone–subunit complexes [40,46,47], consistent with the NTD being the initial recruitment site.

Previously, only chaperone–subunit complexes were found to bind to the usher, not the free chaperone [48,49]. Because the usher almost exclusively interacts with the chaperone of the chaperone–subunit complex [45,50], recruitment of solely a chaperone without its subunit cargo is avoided by a so-called proline lock within the usher binding surface (UBS) of the chaperone: the structure of a chaperone without cargo shows a pair of conserved proline residues at the beginning of the subunit-binding F1G1 loop that occlude the usher-binding surface and prevent the usher from binding. In a chaperone–subunit complex, the proline lock rotates away from the usher-binding surface, allowing the usher–chaperone–subunit complex to form [34].

In this context, another crucial question is how the usher is able to discern which subunit to start the assembly process with and in which order to incorporate the subsequent different subunits, because there is only minimal interaction between the NTD and the different pilin subunits.

Initial work on the type 1 pilus assembly suggested a possible basis for subunit ordering: the ordered region of the usher NTD (aa 25–125) interacts only with the chaperone, the disordered region (aa 1–24) folds upon binding to the subunit in the chaperone–subunit complex and could be therefore responsible for the discrimination of the different FimC–subunit complexes by the assembly platform [45]. Indeed, FimD binds to different FimC–subunit complexes with different affinities, which would be a key element for correct initiation of pilus assembly and for the correct ordering of the subunit incorporation into the pilus. Consistent with this is the fact that, among all chaperone–subunit complexes, the chaperone–adhesin complex exhibits the highest affinity for the usher NTD [49]. However, the rank order of NTD-binding affinities for the other tip fibrillum subunits does not match the incorporation order as observed in the mature pilus [40,45–47], suggesting that other factors play a role (see below).

Di Yu et al. [34] determined the surface of the adhesin in contact with the NTD in the usher NTD–chaperone–subunit complex of the F1 pilus system to be only 3% of the total surface and argue that this is not significant enough to determine the subunit ordering. Instead, an alternative subunit ordering mechanism is suggested: the interaction with the different subunits affects the conformation of the chaperone’s UBS in a differentiated manner, resulting in different affinities of the chaperone towards the usher NTD depending on the subunit it is in DSC with.
Figure 3. Crystal structures of the usher pore domain in two different conformations: the inactive apo usher and the active open usher. The side (top) and extracellular (bottom) views of the usher pore domain (in blue except for loop 220–232 and helix 443–454 in yellow and orange, respectively, relevant in usher gating [61]) are represented for the apo usher (left, PDB code 3OHN) and the active usher (right, PDB code 3RFZ). The Cα–Cα dimensions are indicated. The plug domain (pink) occludes the pore in the apo usher, whereas it is displaced towards the periplasmic space in the active usher. (Online version in colour.)

Comparison of the structures of the different chaperone–subunit complexes available (PapA, PapG, PapH and FimA, FimF, FimH) [30,31,38,47,51–54], indeed, shows a significantly different conformation in the UBS between those chaperone–subunit complexes binding weakly (PapA, PapH, FimA) and those binding more tightly (PapG, FimF, FimH) to the usher NTD. Thus, subtle conformational changes in the chaperone could therefore modulate the affinity towards the usher [34].

However, pilus subunit ordering is not solely determined by the affinities of the chaperone–subunit complexes for the usher’s NTD but depends also on the DSE rate between subunits. In vitro studies have been carried out on the DSE rates between cognate and non-cognate subunit pairs in both the Fim and the Pap systems and both with and without usher [55–57]. By ‘cognate’, we mean here pairs of adjacent subunits interacting with each other as observed in the mature pilus. From these results, it became evident that DSE rates between cognate pairs are much higher than between non-cognate pairs (two- to 50-fold), whereby the DSE rates followed the expected trend according to the established incorporation order in the tip fibrillum: for example, in the presence of an activated usher, FimG undergoes clearly the fastest DSE with FimH (171 min$^{-1}$), followed by FimF with FimG (3 min$^{-1}$), then FimA with FimF (0.03 min$^{-1}$). It is interesting to note that, once the tip fibrillum is assembled in the correct order, the DSE of FimA–FimA is extremely high (960 min$^{-1}$) [55,57].

Finally, another factor which must influence the order in which subunits are incorporated within the pilus is the concentration of the different chaperone–subunit complexes in the periplasm [57,58]. Using the known usher-mediated DSE rate [55,57] and the known affinities of the various subunits for the usher NTD [46], a comprehensive mathematical model of pilus biogenesis was generated [57]. It was shown that the mathematically derived ordering of pilus subunit displays a significant dependence on chaperone–subunit concentrations. For example, a correctly ordered pilus tip could only be obtained if a concentration of FimC–FimG in 10-fold excess over that of FimC–FimF was assumed. Unfortunately, the concentrations of the various chaperone–subunit complexes within the periplasm during pilus biogenesis are unknown and remain to be determined.

Although the FimC–FimH complex is known to be needed to activate the usher [49,59,60], the usher activation mechanism is still unclear. One major step in usher activation is the swinging
Figure 4. Model of subunit incorporation cycle by the chaperone–usher pathway. Based on the type 1 pilus system (colour codes of the usher and subunit structures are based on figure 1). The apo usher (view (a)) is occluded by its plug domain. The recruitment of the FimC–FimH complex (step 1) at the usher NTD (view (b)) initiates usher activation. FimC–FimH is subsequently inserted inside the usher pore domain (step 2) and transferred to the usher CTDs (view (c)). Next, subunit FimG is recruited at the usher NTD (step 3, view (d)). FimH undergoes DSE with FimG Nte (step 4), releasing the chaperone of FimH (view (e)). FimG is then transferred to the usher CTDs, whereas the pilin domain of FimH translocates through the pore (step 5). The FimD–FimC–FimG–FimH complex (view (f)) is then ready for the next round of subunit recruitment (step 6) with FimC–FimF binding to the usher NTD (view (g)). DSE with FimF Nte (step 7) releases the chaperone of FimG (view (h)). FimF transfers to the CTDs, whereas the pilin domain of FimG enters the pore (step 8), exposing the entire adhesin FimH to the extracellular milieu (view (i)). The FimD–FimC–FimF–FimG–FimH complex (view (i)) is then ready for the cyclic incorporation of the major subunit FimA (step 9). (Online version in colour.)

out of the plug from its position within the usher pore lumen to its position in the periplasm where it interacts with the usher NTD. Electrophysiological studies suggested that both the NTD and the CTDs are involved in gating the usher, even in the absence of a translocation substrate [61], with only the FimH adhesin being able to stabilize the plug–NTD interaction in the open state while reaching out for the pore [43,47] (figure 4, view (b)). Recently, it was proposed that the lectin domain of FimH actively displaces the plug domain, because the plug domain was calculated to have weaker interactions with the usher lumen than the lectin domain of FimH [27]. Deletion of the plug domain showed that it is essential in pilus biogenesis [61,62], its role in active subunit recruitment becoming recently apparent: the NTD of PapC alone was only able to bind PapD–PapG and PapD–PapE, but not the other chaperone–subunit complexes PapD–PapK/A/H, whereas an NTD–plug complex is able to bind all chaperone–subunit complexes [47].

(b) The CTD's role: pilus elongation

Early biochemical studies identified a complex of FimC–FimH with the CTDs of FimD [49], later confirmed by the high-resolution structure of the ternary complex FimD–FimC–FimH [43]
Thus, after activation and initial recruitment of FimC–FimH to the usher NTD, the FimC–FimH complex must be transferred to the CTDs (figure 4, step 2).

How this ‘handover’ is managed is still not completely clear, although it was shown that CTD2 is able to destabilize the NTD–FimC–FimH complex, increasing the dissociation rate by one order of magnitude [47]. Except for the terminator complex PapD–PapH, CTD2 was able to bind to all chaperone–subunit complexes, although with much lower affinity than the NTD or the NTD–plug complex [47], which is in line with it being the second station for subunits during pilus growth.

Superimposition of the structure of the FimD NTD bound to the FimC–FimF complex [50] with the structure of the full-length FimD bound to the FimC–FimH complex—using the NTD structure in both as a guide for superimposition—revealed that chaperone–subunit complexes could be accommodated at the same time at the NTD- and the CTD-binding site(s) without steric clashes (figure 4, view c). Moreover, the Nte of the subunit in the chaperone–subunit complex bound at the usher NTD is oriented towards the receiving groove of the chaperone–subunit complex bound at the usher CTDs, the NTD-bound subunit’s P5 residue being located right next to the P5 pocket of the CTD-bound subunit (figure 4, views d, g, j). Thus, the two subunits are ready to undergo DSE. This and other results led us to propose a general subunit-incorporation cycle mechanism by a monomeric usher that is shown in figure 4 [43,57].

The high-resolution structure of FimD–FimC–FimF–FimG–FimH [27] (figure 4, view i) captures another remarkable snapshot of a more advanced stage in pilus biogenesis—the full assembly of the tip fibrillum of the type 1 pilus (figure 1): the adhesin FimH, at the distal end, is found on the extracellular side of the usher FimD. FimG is in the process of translocation through the usher lumen, and FimF is still in DSC interaction with its chaperone FimC and bound to the CTDs on the periplasmic side of the usher. Thus, this visualizes the three main stages of subunit localization and interaction with its assembly catalyst: in the periplasm, after DSE traversing the OM through the usher, and in the extracellular milieu.

(c) Translocation through the usher

By comparing the FimD–FimC–FimH and FimD–FimC–FimF–FimG–FimH structures, two large conformational changes were identified within FimH. In the FimD–FimC–FimH complex, where the lectin domain of FimH (FimHL) is inside the FimD pore and the pilin domain of FimH (FimHP) is in DSC with FimC and bound to the usher CTDs, the two FimH domains are aligned (i.e. making an angle of 180°) [43]. In the FimD–FimC–FimF–FimG–FimH complex, where both FimH domains are outside on the extracellular milieu side of the pore, the angle between the two domains decreases by 37.5° and the FimHL domain changes from a compressed and translocation-competent state to a decompressed and receptor-competent state. The fact that FimH adopts a more angled shape led to the suggestion that the conformational change acts as a lock to prevent backsliding of FimH into the periplasm and that translocation might be made unidirectional by conformational changes within subunits as the nascent pilus emerges from the usher pore [27]. Interestingly, the major pilin subunit (FimA in type I pili and PapA in P pili) is known to adopt a superhelical structure of about 3.3 subunits per turn as the FimA or PapA polymer exits the usher pore to form the pilus rod [16]. Rod formation at the usher exit would also be able to prevent backsliding during pilus elongation.

But where does the driving force for the translocation step originate from? Geibel et al. [27] (figure 4, view i) recently described a low-energy exit path within the lumen of the FimD pore, which imposes on the subunits a combined translation/rotation which is of the same magnitude as the translation/rotation that chaperone–subunit complexes must be subjected to when transferring from the usher NTD to the usher CTDs, i.e. a 53 Å translation and a 110–120° anticlockwise rotation (figure 4, steps 2, 5 and 8). The low-energy pathway by itself might be a very elegant way of minimizing energetic costs while translocation occurs and certainly would facilitate NTD-to-CTD transfer. However, it is unlikely to provide the energy source required for translocation. Instead, we envisage two distinct but successive steps leading to translocation: (i) the release of the chaperone–subunit complex from the usher NTD caused
by the action of CTD2 after DSE has occurred [47,63] and (ii) the pulling force emanating from super-helix formation of the pilus rod [64]. Given the stability of the super-helix and the weak forces maintaining subunits such as FimG within the pore (as determined in [27]), this seems indeed plausible.

Finally, P pilus assembly was described to come to an end when the termination unit PapH is incorporated into the pilus and thanks to its missing P5 pocket is unable to undergo DSE. Additionally, the usher CTD2 has been shown to bind to all chaperone–subunit complexes except to the chaperone–PapH complex, suggesting that the failure of the handover from NTD to the CTDs is also involved in the termination process [47]. No PapH-like subunit has been identified for the type 1 pilus system and thus termination of pilus biogenesis for this system is still unclear.

4. Conclusion

Using a combination of structural, genetic, thermodynamic and kinetic approaches, unprecedented insights on pilus biogenesis by the chaperone–usher pathway have been obtained. We have now acquired details of almost every step in the process and can even suggest how this highly dynamic process might look (for a molecular animation video of the pilus assembly process, see http://www.youtube.com/watch?v=WQ_g1z8rJY0&feature=youtu.be (valid at the time of writing)).

Nonetheless, a few fascinating questions remain to be answered. Firstly, the adhesins are known to have a role in activating the usher. However, we do not yet know what the roles of the periplasmic domains of the usher in this process are (figure 4, view a), how they assist in the initial translocation process; only the structural rearrangement of the translocation domain from its apo/inactive to its active state could be determined so far. Secondly, how do the NTD and CTD domains rearrange during the handover? What are the concentrations of the various subunits in the periplasm? We indeed know DSE rates and affinities for each interaction partner in the system, but we do not known their concentrations. Notably, we do not know the concentration of the termination subunit, an essential factor in determining pilus average length.

It has been shown that bacterial pathogens deficient in type I or P pili are strongly attenuated in their ability to cause UTIs [65]. A major aim of studying the nanomachines involved in bacterial pathogenesis is to exploit the detailed structural knowledge to move from the current non-specific antibiotic treatments causing growing problems with resistance and therefore with chronic infections to more specific treatments targeting virulence factors. The development of such antibiotics against pili is still in its infancy, because the structure determination of membrane proteins is still a challenging though quickly growing field. However, there have been some successful attempts to inhibit pilus formation and these follow two main strategies, as reviewed in [66]: (i) competitive inhibition of the adhesive structures such as the mannosides [67,68] and globosides [69] and (ii) inhibition of the assembly pathway. The pilicides developed in Pinkner et al. [70] and Chorell et al. [71] directly target the UBS—the surface of the chaperone interacting with the NTD, where the proline lock is located. Other pilicides target the interaction between the chaperone and the subunit [72]. These works emphasize how important it is to gain detailed molecular knowledge. In this context, another attractive target for inhibition is the P5 pocket, which, if made unavailable, could prevent the targeting of the incoming subunit’s Nte to the previously assembled subunit’s groove [73]. We anticipate promising results coming through in the next few years tackling bacterial ‘hair’ growth from a rational, knowledge-based perspective.

**Funding statement.** This work was supported by Long-Term Fellowships from the European Molecular Biology Organization (EMBO, ALTF 745-2009) and Marie Curie FP7-PEOPLE-2009-IEF (252616) to A.B., and by grant 85602 from the Medical Research Council (MRC) to G.W.
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