P27Kip1

Subjects: Cell Biology
Contributor: Debora BENCIVENGA

The Cyclin-dependent kinase (CDK) regulator p27Kip1 is a gatekeeper of G1/S transition. It also regulates G2/M progression and cytokinesis completion, via CDK-dependent or -independent mechanisms. Recently, other important p27Kip1 functions have been described, including the regulation of cell motility and migration, the control of cell differentiation program and the activation of apoptosis/autophagy.

Keywords: p27 ; intrinsically unstructured protein ; scaffold protein ; CDK ; cyclin ; cytoskeleton ; Rho GTPase ; αTAT1

1. A Premise: Unfolding and Scaffolding Might Be Two Strictly Connected Features of Proteins

Different disciplines, like Mathematics, Biology, Genetics, Fundamental Physics, Astronomy and Philosophy, have pointed out the advantage of chaos as opposed to the Newtonian cosmos with its deterministic and linear order [1][2][3]. Indeed, the stochastic and chaotic features of life represent an immense advantage either for facilitating the long way of evolution or for allowing rapid environmental adaptations. In Biochemistry, the rigid protein structure/function paradigm has been progressively abandoned and, in the last twenty years, the discovery of proteins with extensive intrinsically disordered regions has gradually taken place. Partially or totally disordered proteins do not adopt a unique and rigid tridimensional structure and show a high grade of conformational plasticity. In turn, these apparently “chaotic” regions/proteins strongly increase the number of adaptive interactions extending the assortment of their molecular targets. Since ductility characterizes more than half of the human proteome, it can be reasonably assumed that IUPs/IUPRs (Intrinsically Unstructured Proteins/Intrinsically Unstructured Protein Regions) are involved in almost all basic and regulatory processes, accomplishing pivotal tasks in signal transduction, transcription, metabolic control and cell cycle [4][5][6][7]. IUPs, despite the lack of a 3D structure, are morphing over time and might transiently fold to adopt multiple conformations and to work properly. It has been argued that there is no direct evidence of their unstable state in the cellular environment, where, differently from the in vitro experimental conditions, the protein concentration is incredibly high. Thus, some concepts on IUPs that have found confirmation in vitro could not be completely reliable in vivo and IUPs plasticity could be, at least in part, hypothetical. Alternatively, it is conceivable that in vivo the different microenvironments of the various cellular compartments might provide specific interactors and conditions for proper folding in a process known as “folding upon binding” [8][9]. On these bases, the family of IUPs, rather than being defined as a pool of unstructured proteins, could be described as a class of macromolecules capable of rapidly challenging and modifying their three-dimensional architecture, thus undergoing a continuous refolding. This suggests, in turn, that the structure/function axiom may be still valid if adapted to the view that a single protein may give rise to multiple structure/function pairs, with even strongly diverging activities.

To date, many methods, including complex bioinformatic assessments, confirm that the lack of stable protein organization is an intrinsic property of the proteome, with its levels increasing during evolution (> 40% of the proteome in Eukarya branches) [10]. On these bases, a new proteome world has been proposed and called “disorderome”. Indeed, the impressive cell complexity in higher eukaryotes has been achieved using parsimonious strategies in which unfolded domains represent the most suitable method for network rewiring. Specifically, by means of modulable structural states of natively unstructured macromolecules, cells managed to use a limited set of proteins to carry out a fine control of different cellular processes.

An extensive structural plasticity has also been observed in Scaffold Proteins (SPs) (also interchangeably defined Anchor, Adaptor or Docking Proteins) that play important roles in numerous cellular processes. Mostly, these proteins employ their modular structured domains, often in combination with the regions that do not assume stable secondary structures [11]. In the past, there have been many restrictions and limitations in recognizing and identifying novel SPs. The common mode of action of classical SPs involves their ability to connect functionally components of specific protein networks that act in signaling and/or metabolic pathways by increasing their effective concentration, or orienting the interacting partners in a conformation compatible with the cellular context, and/or by limiting their translational movements [12]. SPs are involved

not only in signaling conduits, but also in assembly-line processes, in the control of enzymatic activities and in the transport of bound proteins (cargo function) to a precise cellular compartment [11][13]. They might also exert allosteric control over their partners and might be themselves the target of regulation. Overall, these definitions are unceasingly remolded, and new approaches are continuously introduced to predict candidate proteins which may act as scaffolds [14]. There is the possibility that anchor proteins may use both their structured domains and IUPRs in functional domain–domain interactions that could build up platforms and organizers of protein–protein interaction [15].

In the following, we aim to discuss experimental proofs sustaining the view that p27 Kip1 (hereinafter, p27), a key cell cycle modulator and a well-recognized IUP, might be also envisioned as an SP.

2. A Premise: Unfolding and Scaffolding Might Be Two Strictly Connected Features of Proteins

Different disciplines, like Mathematics, Biology, Genetics, Fundamental Physics, Astronomy and Philosophy, have pointed out the advantage of chaos as opposed to the Newtonian cosmos with its deterministic and linear order [1][2][3]. Indeed, the stochastic and chaotic features of life represent an immense advantage either for facilitating the long way of evolution or for allowing rapid environmental adaptations. In Biochemistry, the rigid protein structure/function paradigm has been progressively abandoned and, in the last twenty years, the discovery of proteins with extensive intrinsically disordered regions has gradually taken place. Partially or totally disordered proteins do not adopt a unique and rigid tridimensional structure and show a high grade of conformational plasticity. In turn, these apparently “chaotic” regions/proteins strongly increase the number of adaptive interactions extending the assortment of their molecular targets. Since ductility characterizes more than half of the human proteome, it can be reasonably assumed that IUPs/IUPRs (Intrinsically Unstructured Proteins/Intrinsically Unstructured Protein Regions) are involved in almost all basic and regulatory processes, accomplishing pivotal tasks in signal transduction, transcription, metabolic control and cell cycle [4][5][6][7]. IUPs, despite the lack of a 3D structure, are morphing over time and might transiently fold to adopt multiple conformations and to work properly. It has been argued that there is no direct evidence of their unstable state in the cellular environment, where, differently from the in vitro experimental conditions, the protein concentration is incredibly high. Thus, some concepts on IUPs that have found confirmation in vitro could not be completely reliable in vivo and IUPs plasticity could be, at least in part, hypothetical. Alternatively, it is conceivable that in vivo the different microenvironments of the various cellular compartments might provide specific interactors and conditions for proper folding in a process known as “folding upon binding” [8][9]. On these bases, the family of IUPs, rather than being defined as a pool of unstructured proteins, could be described as a class of macromolecules capable of rapidly challenging and modifying their three-dimensional architecture, thus undergoing a continuous refolding. This suggests, in turn, that the structure/function axiom may be still valid if adapted to the view that a single protein may give rise to multiple structure/function pairs, with even strongly diverging activities.

To date, many methods, including complex bioinformatic assessments, confirm that the lack of stable protein organization is an intrinsic property of the proteome, with its levels increasing during evolution (> 40% of the proteome in Eukarya branches) [10]. On these bases, a new proteome world has been proposed and called “disorderome”. Indeed, the impressive cell complexity in higher eukaryotes has been achieved using parsimonious strategies in which unfolded domains represent the most suitable method for network rewiring. Specifically, by means of modulable structural states of natively unstructured macromolecules, cells managed to use a limited set of proteins to carry out a fine control of different cellular processes.

An extensive structural plasticity has also been observed in Scaffold Proteins (SPs) (also interchangeably defined Anchor, Adaptor or Docking Proteins) that play important roles in numerous cellular processes. Mostly, these proteins employ their modular structured domains, often in combination with the regions that do not assume stable secondary structures [11]. In the past, there have been many restrictions and limitations in recognizing and identifying novel SPs. The common mode of action of classical SPs involves their ability to connect functionally components of specific protein networks that act in signaling and/or metabolic pathways by increasing their effective concentration, or orienting the interacting partners in a conformation compatible with the cellular context, and/or by limiting their translational movements [12]. SPs are involved not only in signaling conduits, but also in assembly-line processes, in the control of enzymatic activities and in the transport of bound proteins (cargo function) to a precise cellular compartment [11][13]. They might also exert allosteric control over their partners and might be themselves the target of regulation. Overall, these definitions are unceasingly remolded, and new approaches are continuously introduced to predict candidate proteins which may act as scaffolds [14]. There is the possibility that anchor proteins may use both their structured domains and IUPRs in functional domain–domain interactions that could build up platforms and organizers of protein–protein interaction [15].

In the following, we aim to discuss experimental proofs sustaining the view that p27 Kip1 (hereinafter, p27), a key cell cycle modulator and a well-recognized IUP, might be also envisioned as an SP.

3. p27, a Platform Where Assembling Different Complexes and Cellular Structures

It is important to stress, however, that the importance of R194 has been experimentally validated by demonstrating that a peptide (180–194 of p27 sequence) including R194 is a better substrate of CyclinA/CDK2 than a similar peptide with alanine in 194 position [16]. Thus, further studies are clearly necessary for confirming the precise role of R194 in the ternary Cyclin/CDK/p27 complexes.

Besides being a CDK regulator, p27 has been straightforwardly demonstrated to exert important roles in controlling cytoskeleton assembly/disassembly and cell movement. However, although the importance of the protein in these phenomena is functionally well established, the mechanistic details of p27 activity and its precise interactors do not appear definitely identified.

Three major classes of elements, differing in size and in protein composition, work together to form a cytoskeleton of eukaryotic cells [17]. Differently from intermediate filaments, made of different proteins, which have a very rigid structure inside the cells, actin microfilaments and microtubules have very dynamic structures. Actin filaments, organized in the form of meshworks or bundles of parallel fibers, contribute, together with the associated actin-binding proteins, to determine the cell shape and substrate adherence. Being continuously remodeled, they influence cell movements and cytokinesis during mitosis. Microtubules are highly dynamic long cytoplasmic polymers of tubulin [18] required for maintaining cell shape [19] and for the organization of mitotic spindle in dividing cells governing proper separation of sister chromatids and cytokinesis; they also are structural and functional constituents of cilia and flagella [20] and represent a structural platform for the intracellular movement of cellular organelles and for transport of vesicles. Furthermore, these structures are important for the assembly of macromolecules including enzymes and structural proteins and for facilitating the organization of pathways.

During neurogenesis, cell cycle exit, cell migration and neuronal differentiation must be harmonized and, apparently, they are linked by p27 activities played in proliferation control [21][22], MTs' dynamic regulation [23] and stabilization of Neurogenin 2 [24]. The evidence of p27 N-terminal half in the Neurogenin 2 binding and stabilization represents an additional CDK- and cell cycle-independent activity played in the neuronal differentiation contributing to the cortex development and confirms p27 scaffolding role in the neurogenic program.

4. Perspectives and Conclusions. p27, Human Diseases and Drug Design

In the past years, compelling evidence has demonstrated the importance of p27 (both as level and activity) in numerous human diseases, mainly in human cancers. The first observation of a possible role of p27 in cancerogenesis stems from a seminal study on mice ablated of the Cdkn1b gene [25]. These genetically modified animals, in addition to showing an increased body size and hyperplasia of several organs, developed pituitary adenomas [25]. Furthermore, Cdkn1b +/− mice were more susceptible than normal animals to the development of cancer due to chemical carcinogens or irradiation [26]. Some years later, it was shown that rats with a homozygous alteration of Cdkn1b had a peculiar form of MEN, called MENX [27]. Pellegata and collaborators have also described the spontaneous development of MENX in rats hemizygous for Cdkn1b although with a lower frequency with respect of homozygous animals [28]. The finding strongly reinforces the view of Cdkn1b as a haploinsufficient tumor suppressor gene in mouse and rats. Numerous findings have also corroborated the opinion that p27 alterations are important in human carcinogenesis. Indeed, p27 decrease has been demonstrated in breast, colon, prostate and ovarian cancers [29]. Moreover, tumors with p27 reduction also showed increased aggressiveness. The molecular basis of p27 decrease has been, at least initially, associated with an enhanced degradation or with a mislocalization of the protein in the cytosol [29]. More recently, due to the development of novel technological approaches, i.e., Next Generation Sequencing and Genome-wide Association Analyses, CDKN1B has been identified as mutated in several cancers. In luminal breast cancers, CDKN1B constitutes one of the 18 genes most frequently altered [30]. Moreover, in this frequent breast cancer type, the downregulation of p27 causes resistance to anti-HER2 therapies and radiotherapies [31]. CDKN1B has also been found frequently altered in prostate cancers and in hairy cell leukemia where it represents the second most altered gene after BRAF [32]. Finally, in sporadic parathyroid adenomas, in small intestine neuroendocrine tumors [33][34][35] and in MEN4 (a new MEN subtype), CDKN1B is recurrently altered [36]. All these finding definitely demonstrated a role of p27 in human cancerogenesis and/or in tumor evolution.

p27 alterations have also been demonstrated in a specific condition associated with human diseases. Indeed, in endometriosis, p27 appears significantly reduced, and in congenital eye alteration, p27 post-synthetic changes have been demonstrated [37][38]. Haploinsufficiency of CDKN1B was demonstrated to improve cardiac function in mice post-myocardial infarction [39]. Finally, a role of p27 in subarachnoid hemorrhage has been reported [40]. Based on all this evidence, the possibility of targeting p27, either positively or negatively, appears of pharmacological interest.

A recent study by Kriwaki's group reported the possibility of affecting the binding of p27 with other proteins [41]. The study was performed with the goal of reducing p27 cytosolic carcinogenetic properties. Through NMR-based screening, small molecules that bound p27 specifically, albeit with low affinity, in regions containing aromatic rings (with predilection for amino acids W60/76 and Y88/89) of the kinase inhibitory domain were identified. The authors also demonstrated that one of the tested compounds sequesters p27 from Cyclin A/CDK2, binding its D2 domain, a p27 region that specifically interacts with CDKs and folds upon this binding. The drug-dependent p27 displacement restores CDK2 catalytic activity, demonstrating the pharmacological effect of this small molecule, able to block p27 function. Despite this effect being associated with cancer development, the proposal of the Kriwacki group demonstrated how it will be possible to drug transient unfolded protein-folded protein interfaces [41].

Why are these studies promising? Since p27 might be considered in some instances an SP, the Kriwaki study suggests that proteins facilitating the formation of complexes, albeit unstructured, might be the target of therapy, allowing the conclusion that the knowledge of the region involved in specific binding, even if part of IUP, might be considered for efficacious therapy. Obviously, their relevance depends on a detailed knowledge of interactors of the protein region to be targetable.

References

1. Toker, D.; Sommer, F.T.; D'Esposito, M. A simple method for detecting chaos in nature. Commun. Biol. 2020, 3, 1–13.
2. Stark, J.; Hardy, K. Chaos: Useful at Last? Science 2003, 301, 1192–1193.
3. Newton, I.S. Philosophiae Naturalis Principia Mathematica, 1st ed.; Streater, J., Ed.; Royal Society of London: London, UK, 1687.
4. Wright, P.E.; Dyson, H.J. Intrinsically disordered proteins in cellular signalling and regulation. Nat. Rev. Mol. Cell Biol. 2015, 16, 18–29.
5. Minezaki, Y.; Homma, K.; Kinjo, A.R.; Nishikawa, K. Human transcription factors contain a high fraction of intrinsically disordered regions essential for transcriptional regulation. J. Mol. Biol. 2006, 359, 1137–1149.
6. Protter, D.S.W.; Parker, R. Principles and Properties of Stress granules. Trends Cell Biol. 2016, 26, 668–679.
7. Galea, C.A.; Wang, Y.; Sivakolundu, S.G.; Kriwacki, R.W. Regulation of Cell Division by Intrinsically Unstructured Proteins; Intrinsic Flexibility, Modularity and Signaling Conduits. Biochemistry 2008, 47, 7598–7609.
8. Sugase, K.; Dyson, H.J.; Wright, P.E. Mechanism of coupled folding and binding of an intrinsically disordered protein. Nature 2007, 447, 1021–1025.
9. Robustelli, P.; Piana, S.; Shaw, D.E. Mechanism of Coupled Folding-upon-Binding of an Intrinsically Disordered Protein. J. Am. Chem. Soc. 2020, 142, 11092–11101.
10. Piovesan, D.; Tabaro, F.; Mičetić, I.; Necci, M.; Quaglia, F.; Oldfield, C.J.; Aspromonte, M.C.; Davey, N.E.; Davidović, R.; Dosztányi, Z.; et al. DisProt 7.0: A major update of the database of disordered proteins. Nucleic Acids Res. 2017, 45, 219–227.
11. Good, M.C.; Zalatan, J.G.; Lim, W.A. Scaffold proteins: Hubs for controlling the flow of cellular information. Science 2011, 332, 680–686.
12. Langeberg, L.K.; Scott, J.D. Signalling scaffolds and local organization of cellular behavior. Nat. Rev. Mol. Cell Biol. 2015, 16, 235–244.
13. Shaw, A.S.; Filbert, E.L. Scaffold proteins and immune-cell signalling. Nat. Rev. Immunol. 2009, 9, 47–56.
14. Oh, K.; Yi, G.S. Prediction of scaffold proteins based on protein interaction and domain architectures. BMC Bioinform. 2016, 17 (Suppl. S6), 220.
15. Reed, B.J.; Locke, M.N.; Gardner, R.G. A conserved deubiquitinating enzyme uses intrinsically disordered regions to scaffold multiple protein interaction sites. J. Biol. Chem. 2015, 290, 20601–20612.
16. Li, J.; Vervoorts, J.; Carloni, P.; Rossetti, G.; Lüscher, B. Structural prediction of the interaction of the tumor suppressor p27Kip1 with cyclin A/CDK2 identifies a novel catalytically relevant determinant. BMC Bioinform. 2017, 18, 15.

17. Hohmann, T.; Dehghani, F. The Cytoskeleton-A Complex Interacting Meshwork. Cells 2019, 8, 362.

18. Desai, A.; Mitchison, T.J. Microtubule polymerization dynamics. Annu. Rev. Cell Dev. Biol. 1997, 13, 83–117.

19. Rodriguez, O.C.; Schaefer, A.W.; Mandato, C.A.; Forscher, P.; Bement, W.M.; Waterman-Storer, C.M. Conserved microtubule-actin interactions in cell movement and morphogenesis. Nat. Cell Biol. 2003, 5, 599–609.

20. Loreng, T.D.; Smith, E.F. The Central Apparatus of Cilia and Eukaryotic Flagella. Cold Spring Harb. Perspect. Biol. 2017, 9, a028118.

21. Fero, M.L.; Rivkin, M.; Tasch, M.; Porter, P.; Carow, C.E.; Firpo, E.; Polyak, K.; Tsai, L.H.; Broudy, V.; Perlmutter, R.M.; et al. A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. Cell 1996, 85, 733–744.

22. Soppa, U.; Schumacher, J.; Florencio Ortiz, V.; Pasqualon, T.; Tejedor, F.J.; Becker, W. The Down syndrome-related protein kinase DYRK1A phosphorylates p27(Kip1) and Cyclin D1 and induces cell cycle exit and neuronal differentiation. Cell Cycle 2014, 13, 2084–2100.

23. Godin, J.D.; Thomas, N.; Laguesse, S.; Malinouskaya, L.; Close, P.; Malaise, O.; Purnelle, A.; Raineteau, O.; Campbell, K.; Fero, M.; et al. p27(Kip1) is a microtubule-associated protein that promotes microtubule polymerization during neuron migration. Dev. Cell 2012, 23, 729–744.

24. Nguyen, L.; Besson, A.; Heng, J.I.; Schuurmans, C.; Teboul, L.; Parras, C.; Philpott, A.; Roberts, J.M.; Guillemot, F. p27Kip1 independently promotes neuronal differentiation and migration in the cerebral cortex. Genes Dev. 2006, 20, 1511–1524.

25. Godena, V.K.; Brookes-Hocking, N.; Moller, A.; Shaw, G.; Oswald, M.; Sancho, R.M.; Miller, C.C.; Whitworth, A.J.; De Vos, K.J. Increasing microtubule acetylation rescues axonal transport and locomotor deficits caused by LRRK2 Roc-COR domain mutations. Nat. Commun. 2014, 5, 5245.

26. Fero, M.L.; Randel, E.; Gurley, K.E.; Roberts, J.M.; Kemp, C.J. The murine gene p27Kip1 is haplo-insufficient for tumour suppression. Nature 1998, 396, 177–180.

27. Pellegata, N.S.; Quintanilla-Martinez, L.; Siggelkow, H.; Samson, E.; Bink, K.; Höfler, H.; Fend, F.; Graw, J.; Atkinson, M.J. Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. Proc. Natl. Acad. Sci. USA 2006, 103, 15558–15563.

28. Molatore, S.; Kügler, A.; Irmler, M.; Wiedemann, T.; Neff, F.; Feuchtinger, A.; Beckers, J.; Robledo, M.; Roncaroli, F.; Pellegata, N.S. Characterization of neuroendocrine tumors in heterozygous mutant MENX rats: A novel model of invasive medullary thyroid carcinoma. Endocr. Relat. Cancer 2018, 25, 145–162.

29. Bencivenga, D.; Caldarelli, I.; Stampone, E.; Mancini, F.P.; Balestrieri, M.L.; Della Ragione, F.; Borriello, A. p27Kip1 and human cancers: A reappraisal of a still enigmatic protein. Cancer Lett. 2017, 403, 354–365.

30. Stephens, P.J.; Tarpey, P.S.; Davies, H.; Van Loo, P.; Greenman, C.; Wedge, D.C.; Nik-Zainal, S.; Martin, S.; Varela, I.; Bignell, G.R.; et al. The landscape of cancer genes and mutational processes in breast cancer. Nature 2012, 486, 400–404.

31. Wander, S.A.; Zhao, D.; Slingerland, J.M. p27: A barometer of signaling deregulation and potential predictor of response to targeted therapies. Clin. Cancer Res. 2011, 1, 12–18.

32. Dietrich, S.; Hüllein, J.; Lee, S.C.; Hutter, B.; Gonzalez, D.; Jayne, S.; Dyer, M.J.; Oleś, M.; Else, M.; Liu, X.; et al. Recurrent CDKN1B (p27) mutations in hairy cell leukemia. Blood 2015, 126, 1005–1008.

33. Francis, J.M.; Kiezun, A.; Ramos, A.H.; Serra, S.; Pedamallu, C.S.; Qian, Z.R.; Banck, M.S.; Kanwar, R.; Kulkarni, A.A.; Karpathakis, A.; et al. Somatic mutation of CDKN1B in small intestine neuroendocrine tumors. Nat. Genet. 2013, 45, 1483–1486.

34. Maxwell, J.E.; Sherman, S.K.; Li, G.; Choi, A.B.; Bellizzi, A.M.; O'Dorisio, T.M.; Howe, J.R. Somatic alterations of CDKN1B are associated with small bowel neuroendocrine tumors. Cancer Genet. 2015, 208, 564–570.

35. Costa-Guda, J.; Marinoni, I.; Molatore, S.; Pellegata, N.S.; Arnold, A. Somatic mutation and germline sequence abnormalities in CDKN1B, encoding p27Kip1, in sporadic parathyroid adenomas. J. Clin. Endocrinol. Metab. 2011, 96, E701–E706.

36. Pellegata, N.S. MENX and MEN4. Clinics (Sao Paulo) 2012, 67 (Suppl. S1), 13–18.

37. Goncalves, G.A. p27kip1 as a key regulator of endometriosis. Eur. J. Obstet. Gynecol. Reprod. Biol. 2018, 221, 1–4.

38. Rowan, S.; Chang, M.L.; Reznikov, N.; Taylor, A. Disassembly of the lens fiber cell nucleus to create a clear lens: The p27 descent. Exp. Eye Res. 2017, 156, 72–78.

39. Zhou, N.; Huan, Q.; Cheng, W.; Ge, Y.; Li, D.; Wang, J. p27kip1 haploinsufficiency preserves myocardial function in the early stages of myocardial infarction via Atg5-mediated autophagy flux restoration. Mol. Med. Rep. 2019, 20, 3840–

3848.

40. Chen, D.; Wang, X.; Huang, J.; Cui, S.; Zhang, L. CDKN1B mediates apoptosis of neuronal cells and inflammation induced by oxyhemoglobin via mir-502-5p after subarachnoid hemorrhage. J. Mol. Neurosci. 2020, 70, 1073–1080.
41. Tsytlonok, M.; Sanabria, H.; Wang, Y.; Felekyan, S.; Hemmen, K.; Phillips, A.H.; Yun, M.K.; Waddell, M.B.; Park, C.G.; Vaithiyalingam, S.; et al. Dynamic anticipation by Cdk2/Cyclin A-bound p27 mediates signal integration in cell cycle regulation. Nat. Commun. 2019, 10, 1676.